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(54) Title: HUMAN MONOCLONAL ANTIBODIES

(57) Abstract

This invention relates to novel human monoclonal antibodies (mAbs) and to the genes encoding same. More specifically, this invention relates to human monoclonal antibodies specifically reactive with an epitope of the fusion (F) protein of Respiratory Syncytial Virus (RSV). Such antibodies are useful for the therapeutic and/or prophylactic treatment of RSV infection in human patients, particularly intants and young children.

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HUMAN MONOCLONAL ANTIBODIES

Field of the Invention:

This invention relates to novel human monoclonal antibodies (mAbs) and to the genes encoding same. More specifically, this invention relates to human monoclonal antibodies specifically reactive with an epitope of the fusion (F) protein of Respiratory Syncytial Virus (RSV). Such antibodies are useful for the therapeutic and/or prophylactic treatment of RSV infection in human patients, particularly infants and young children.

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Background of the Invention:

Respiratory syncytial virus (RSV) is the major cause of lower respiratory disease in children, giving rise to predictable annual epidemics of bronchiolitis and pneumonia in children worldwide. The virus is highly contagious, and infections can occur at any age. Immunity to RSV appears to be short-lived, thus reinfections are frequent. Zero to 2 year old infants are the most susceptible and represent the primary affected population. In this group, 1 out of 5 will develop lower respiratory (below larynx) disease upon infection and this ratio stays the same upon reinfection. Depending on age, environment and other associated factors, hospitalization is required in 1-3% of cases of RSV infection and is usually of long duration (up to 3 weeks). The high morbidity of RSV infection, especially in infancy, has also been implicated in the development of respiratory problems later in life. Mortality is generally very low in more developed countries, but much higher in less developed countries and in certain risk groups such as children with heart/lung disease, making prophylactic treatment desirable for these groups of children.

A vaccine for RSV infection is not currently available. Severe safety issues surrounding an attenuated whole virus vaccine tested in the 1960s, as well as the potential of induced immunopathology associated with the newer candidate subunit vaccines make the prospects of a vaccine in the near future appear remote. To date one drug therapy, Ribavirin, a broad spectrum antiviral, has been approved. Ribavirin has gained only minimal acceptance owing to problems of administration.

mild toxicity and questionable efficacy. In the majority of cases, hospitalized children receive no drug therapy and receive only intensive supportive care which is extremely costly. It is clear that there is a need for a safe, effective and easily administered drug for the treatment of RSV infection.

The feasibility of passive antibody treatment/protection against RSV has been well established using animal models. Most of the earlier passive transfer studies in animals against infectious agents, including RSV, utilized murine mABs. Recently, the FDA has approved for use intravenous gammaglobulins (IVIG) isolated from pooled human sera. Initial reports from this study had been encouraging (Groothuis, J. R. et al., Antimicrob. Agents Chemo. 35(7):1469-1473 (1991)). However, generic shortcomings of IVIGs exist and include, without limitation, the fact that such products are human blood derived and grams of antibody often need to be administered to achieve an effective dose.

Alternatively, monoclonal antibodies have been employed. The advantages of such an approach include: a higher concentration of specific antibody can be achieved thereby reducing the amount of globulin required to be given; the reliance on direct blood products can be eliminated; the levels of antibody in the preparation can be more uniformly controlled and the routes of administration can be extended. While passive immunotherapy employing monoclonal antibodies from a heterologous species (e.g., murine) has been suggested (See: PCT Application PCT/US94/08699, Publication No. WO 95/04081), one alternative to reduce the risk of an undesirable immune response on the part of the patient directed against the foreign antibody is to employ "humanized" antibodies. These antibodies are substantially of human origin, with only the Complementarity Determining Regions (CDRs) being of non-human origin. Particularly useful examples of this approach are disclosed in PCT Application PCT/GB91/01554, Publication No. WO 92/04381 and PCT Application PCT/GB93/00725, Publication No. WO93/20210.

A second and more preferred approach is to employ fully human mAbs.

Unfortunately, there have been few successes in producing human monoclonal antibodies through classic hybridoma technology. Indeed, acceptable human fusion partners have not been identified and murine myeloma fusion partners do not work

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Well with human cells, yielding unstable and low producing hybridoma lines. However, recent advances in molecular biology and immunology make it now possible to isolate human mABs, particularly directed against foreign infectious agents, as explained in greater detail below.

Comprehensive details concerning RSV infection and its clinical features can be obtained from excellent recent reviews by McIntosh, K. and R. M. Chanock, In: "Respiratory Syncytial Virus", Ch. 38, B.N. Fields ed., Raven Press (1990) and Hall, C.B., In: "Textbook of Pediatric Disease" Feigin and Cherry, eds., W.B. Saunders, pgs 1247-1268 (1987). RSV, belonging to the family paramyoxoviridae. is a negative-strand unsegmented RNA virus with properties similar to those of the paramyxoviruses. It has, however been placed in a separate genus Pneumovirus. based on morphologic differences and lack of hemagglutinin and neuraminidase activities. RSV is pleomorphic and ranges in size from 150-300 nm in diameter. The virus matures by budding from the outer membrane of a cell and virions appear as membrane-bound particles with short, closely spaced projections or "spikes". The RNA genome encodes 10 unique viral polypeptides ranging in size from 9.5 kDa to 160 kDa (Huang, Y. T. and G. W. Wertz, J. Virol. 43:150-157 (1982)). Seven proteins (F, G, N, P, L, M, M2) are present in RSV virions and at least three proteins (F, G, and SH) are expressed on the surface of infected cells. The F protein has been conclusively identified as the protein responsible for cell fusion since specific antibodies to this protein inhibit syncytia formation in vitro and cells infected with vaccinia virus expressing recombinant F protein form syncytia in the absence of other RSV virus proteins. In contrast, antibodies to the G protein do not block syncytia formation but prevent attachment of the virus to cells.

RSV can be divided into two antigenically distinct subgroups, (A & B) (Mufson, M. A. et al., J. Gen'l. Virol. 66:2111-2124 (1985)). This antigenic dimorphism is linked primarily to the surface attachment (G) glycoprotein (Johnson, R. A. et al., Proc. Nat'l. Acad. Sci. USA 84:5625-5629 (1987)). Strains of both group A and B circulate simultaneously, but the proportion of each may vary unpredictably from year to year. An effective therapy must therefore target both subgroups of the

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virus and this is the reason for the selection of the highly conserved surface fusion (F) protein as target antigen for mAb therapy as will be discussed later.

RSV is distributed worldwide. One of the most remarkable features of the epidemiology of RSV virus, as mentioned above, is the consistent pattern of infection and disease. Other respiratory viruses cause epidemics at irregular intervals or exhibit a mixed endemic/epidemic pattern, but RSV is the only respiratory viral pathogen that produces a sizable epidemic every year in large urban centers. In the temperate areas of the world, RSV epidemics have occurred primarily in the late fall, winter or spring but never during the summer. The occurrence and spread of infection within a community is characteristic and easily diagnosed, leading to sharp rises in cases of bronchiolitis and pediatric pneumonia and the number of hospital admissions of young children with acute lower respiratory tract disease. Other respiratory viral agents that occur in outbreaks are rarely present at the same time as RSV. Primary RSV infection occurs in the very young and virtually all children have been infected before they have entered school. By 1 year of age, 25-50% of infants have specific antibodies as a result of natural infection and this is close to 100% by age 4-5. Age, sex, socioeconomic and environmental factors can all influence the severity of disease. With current intensive care in the U.S., overall mortality for normal subjects is low (less than 2% of hospitalized subjects) but can be much higher in infants with underlying cardiac condition (cyanotic congenital heart disease) or respiratory disease (bronchopulmonary dysplasia) where the progression of symptoms may be rapid. For instance, mortality in infants with cyanotic congenital heart disease has been reported to be as high as 37%. In premature infants apneic spells due to RSV infection may occur and, in rare cases, cause neurologic or systemic damage. Severe lower respiratory tract illness (bronchiolitis and pneumonia) is most common in patients under six months of age. Infants who have apparently recovered completely from this illness may display symptomatic respiratory abnormalities for years (recurrent wheezing, decreased pulmonary function, recurrent cough, asthma, and bronchitis).

The mechanisms by which the immune system protects against RSV infection and reinfection are not well understood. It is clear, however, that immunity

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occurs in infants only weeks after recovery from a primary infection. Both serum and secretory antibodies (IgA) have been detected in response to RSV infection in adults as well as in very young infants. However, the titers of serum antibodies to the viral F or G glycoprotein, as well as of neutralizing antibodies found in infants (1-8 months of age) are 15-25% of those found in older subjects. These reduced titers may contribute to the increased incidence of serious infection in younger children.

Evidence for the role of serum antibodies in protection against RSV virus has emerged from epidemiological as well as animal studies. In adults exposed naturally to the virus, susceptibility correlated well with low serum antibody level. In infants, titers of maternally transmitted antibodies correlate with resistance to serious disease (Glezen, W.P. et al., J. Pediatr. 98:708-715 (1981)). Other studies show that the incidence and severity of lower respiratory tract involvement is diminished in the presence of high serum antibody (McIntosh, K. et al., J. Infect. Dis. 138:24-32 (1978)) and high titers of passively administered serum neutralizing antibodies have been shown to be protective in a cotton rat model, of RSV infection (Prince, G. A. et al., Virus Res. 3:193-206 (1985)).

Children lacking cell-mediated immunity are unable to cease their infection and shed virus for many months in contrast to children with normal immune systems. Similarly, nude mice infected with RSV virus persistently shed virus.

These mice can be cured by adoptive transfer of primed T cells (Cannon, M. J. et al., Immunology 62:133-138 (1987)).

In summary, it appears that both cellular and humoral immunity are involved in protection against infection, reinfection and RSV disease and that although antigenic variation is limited, protective immunity is not complete even after multiple exposures.

This invention relates to the use of human mABs specific for the F protein of RSV virus to passively treat or prevent infection. The use of passive antibody therapy in humans is well documented and is being used to treat other infectious diseases such as hepatitis and cytomegalovirus. Clinical trials are also on-going to evaluate the efficacy of humanized antibodies for treatment of RSV infection in

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young children. Studies in animals have clearly demonstrated that polyclonal and monoclonal antibody against both F and G glycoprotein can confer passive protection in RSV virus infection when given prophylactically or therapeutically (Prince, et al., supra). In these studies, passive transfer of neutralizing F or G mAbs to mice, cotton rats or monkeys, significantly reduce or completely prevent replication of the RSV virus in the lungs.

The induction of neutralizing antibodies to RSV virus appears to be limited to the F and G surface glycoproteins. Of these two proteins, the F protein is the major target for cross-reactive neutralizing antibodies associated with protection against different strains of RSV virus. In addition, experimental vaccination of mice or cotton rats with F protein also results in cross protection. The antigenic relatedness of the F protein across strains and subgroups of the virus is reflected in its high degree of homology at the amino acid level. In contrast, in the two subgroups and various strains of RSV, antigenic dimorphism was linked primarily to the G glycoprotein. The F protein has a predicted molecular weight of 68-70 kDa; a signal peptide at its N-terminus; a membrane anchor domain at its C terminus; and is cleaved proteolytically in the infected cell prior to virion assembly to yield disulfide linked F₂ and F₁. Five neutralizing epitopes have been identified within the F protein sequence and map to residues 205-225; 259-278; 289-299: 483-488 and 417-438. Studies to determine the frequency of sequence diversion in the F protein showed that the majority of the neutralizing epitopes were conserved in all of the 23 strains of RSV virus isolated in Australia, Europe, and regions of the U.S. over a period of thirty years. In another study, seroresponses of forty three infants and young children to primary infection with subgroup A or a subgroup B strain showed that responses to homologous and heterologous F antigens were not significantly different, while the G proteins of the subgroup A and B strains were quite unrelated. Moreover, antibody inhibition of virus-mediated cell fusion in vitro versus inhibition of infection correlates best with protection in animal models and fusion inhibition is primarily restricted to F protein specific antibodies. Clearly, the F protein is the more important target for antibody therapy.

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Fully human mAbs to RSV F protein remain a desirable option for the treatment of this disease. Although some success has been reported in obtaining fragments of such mAbs (Barbas, C.F. et al., Proc. Nat'l. Acad. Sci. USA 89:10164-10168 (1992);Crowe, J. E. et al., Proc. Nat'l. Acad. Sci. USA 91: 1386-1390 (1994) and PCT application number PCT/US93/08786, published as WO94/06448, March 31, 1994)), the achievement of such results is not straight forward and novel human mABs as described herein, when and however obtained, are particularly useful alone or in combination with existing molecules to form immunotherapeutic compositions. This invention relates to one such group of human mAbs.

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Brief Description of the Invention:

This invention relates to fully human monoclonal antibodies and functional fragments thereof specifically reactive with an F protein epitope of RSV and capable of neutralizing RSV infection.

In a related aspect, the present invention provides modifications to neutralizing Fab fragments or F(ab')₂ fragments specific for the F protein of RSV produced by random combinatorial cloning of human antibody sequences and isolated from a filamentous phage Fab display library.

In still another aspect, there is provided a reshaped human antibody containing human heavy and light chain constant regions from a first human donor and heavy and light chain variable regions or the CDRs thereof derived from human neutralizing monoclonal antibodies for the F protein of RSV derived from a second human donor.

In yet another aspect, the present invention provides a pharmaceutical composition which contains one (or more) altered antibodies and a pharmaceutically acceptable carrier.

In a further aspect, the present invention provides a method for passive immunotherapy of RSV disease in a human by administering to said human an effective amount of the pharmaceutical composition of the invention for the prophylatic or therapeutic treatment of RSV infection.

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In yet another aspect, the present invention provides methods for, and components useful in, the recombinant production of human and altered antibodies (e.g., engineered antibodies, CDRs, Fab or F(ab)₂ fragments, or analogs thereof) which are derived from human neutralizing monoclonal antibodies (mAbs) for F protein of RSV. These components include isolated nucleic acid sequences encoding same, recombinant plasmids containing the nucleic acid sequences under the control of selected regulatory sequences which are capable of directing the expression thereof in host cells (preferably mammalian) transfected with the recombinant plasmids. The production method involves culturing a transfected host cell line of the present invention under conditions such that the human or altered antibody is expressed in said cells and isolating the expressed product therefrom.

In yet another aspect of the invention is a method to diagnose the presence of RSV in a human which comprises contacting a sample of biological fluid with the human antibodies and altered antibodies of the instant invention and assaying for the occurrence of binding between said human antibody (or altered antibody) and RSV.

In yet another embodiment of the invention is a pharmaceutical composition comprising at least one dose of an immunotherapeutically effective amount of the monoclonal antibody of this invention in combination with at least one additional monoclonal antibody. Especially, when the additional monoclonal antibody is an anti-RSV antibody distinguished from the subject antibody of by virtue of being reactive with a different epitope of the RSV F protein antigen.

Other aspects and advantages of the present invention are described further in the detailed description and the preferred embodiments thereof.

25 <u>Brief Description of the Drawings</u>:

Figure 1 illustrates the cloning strategy used for the construction of the Hu 19A monoclonal antibody. The heavy chain V region was cloned into the PCD derivative vector as a XhoI - Bsp120I fragment. The entire light chain V and C regions were cloned into the PCN derivative vector as a SacI - XbaI fragment. Details are described in the hereinbelow.

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Figure 2 provides a comparison of the heavy chain amino acid sequences of various monoclonal antibodies of this invention. The amino acid sequences of the heavy chains for the A. B. C and D constructs are shown (SEQ ID NOS) 5, 6, 7 and 8, respectively). Numbering of the residues is based on the germline (GL) gene Dp58 (SEQ ID No; 4), beginning at the mature processed amino terminus and ending at CDR3. The "-" indicates identity to the preceding sequence (eg., C compared to B). Sequence A has an amino acid insertion between positions 4 and 5 due to the cloning strategy utilized by Barbas et al. (Proc. Nat'l. Acad. Sci. (USA) 89, 10164-10168 (1992), PCT publication WO94/06448). Bold residues correspond to the leader region, and to CDRs 1-3. The underlined sequence in CDR2 identifies the N-linked glycosylation site in versions A and B that was mutated in version C. Residues P14 and G15, marked with an "*" were listed as L and A, respectively in the published sequence (Barbas et al., supra).

Figure 3 provides a comparison of the light chain amino acid sequences of various monoclonal antibodies of this invention. The amino acid sequences of the light chains for the A, B, C and D constructs are shown (SEQ ID NOS: 10, 11, 12 and 13). Numbering of the residues in the Vκ region is based on the germline (GL) gene Dpk9 (SEQ ID NO: 9), beginning at the mature processed amino terminus and ending at CDR3; but for reference to framework 4, the actual numbering is also shown for Hu19ALc. As in Fig. 2, the "-" indicates identity to the preceding sequence. The G at position 97 in framework 4 of Hu19A, marked with an "*", was listed as E in the published sequence (see text). Sequence A has a two amino acid deletion at residues 1 and 2 due to the cloning strategy. Bold residues correspond to the leader region, and to CDRs 1-3. The κ constant region is shown for constructs A and B in comparison to the germline gene. The L mutation near the C-terminus was corrected in version C (See: Figure 3, SEQ ID NO:13).

Figure 4 illustrates the DNA sequences of plasmids for the expression of the Hu19 mAB heavy and light chains. Figure 4A is the DNA sequence of Hu19AHcpcd (SEQ ID NO:14). The start of translation, leader peptide, aminoterminal processing site (SEQ ID NO:15), carboxy terminus of the 19A heavy chain

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(SEQ ID NO: 16) and *Eco* RI restriction endonuclease cleavage site are shown. Figure 4B is the DNA sequence of Hu19ALcpcn (SEQ ID NO: 17), and shows the corresponding features for the light chain and the *Xba* I restriction site following the end of the coding region for the light chain (SEQ ID NO'S: 18, 19). Figure 4C is the DNA sequence of the coding region of the heavy chain of plasmid Hu19BHcpcd (SEQ ID NO'S 20,21). Figure 4D is the DNA sequence of the coding region for the light chain of plasmid Hu19BLcpcn (SEQ ID NO:22,23 & 24). Figure 4E is the DNA sequence of the coding region of the heavy chain of the plasmid Hu19CHcpcd (SEQ ID NO'S 25,26). Figure 4F is the DNA sequence of the coding sequence of the heavy chain of plasmid Hu19DHcpcd (SEQ ID NO:'S 27,28). Figure 4G is the DNA sequence of the coding region of the light chain of plasmid Hu19CLcpcn (SEQ ID NO'S: 29, 30). In Figures 4C-G, bolded residues indicate differences from the full vector sequences for Hu19AHcpcd and Hu19ALc shown in Figures 4A and 4B, respectively.

Figure 5 illustrates a Coomassie stained SDS-PAGE gel of Hu19B and Hu19C under reducing conditions.

Figure 6 illustrates the separation of Hu19 Glycovarients by anion exchange chromatography.

Figure 7 illustrates SDS-PAGE analysis of Hu19B Fab glycovarients.

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Detailed Description of the Invention:

This invention provides useful human monoclonal antibodies (and fragments thereof) reactive with the F protein of RSV, isolated nucleic acids encoding same and various means for their recombinant production as well as therapeutic, prophylactic and diagnostic uses of such antibodies and fragments thereof.

I. Definitions.

As used in this specification and the claims, the following terms are defined as follows:

"Altered antibody" refers to a protein encoded by an altered immunoglobulin coding region, which may be obtained by expression in a selected host cell. Such

altered antibodies are engineered antibodies (e.g., chimeric, humanized, or reshaped or immunologically edited human antibodies) or tragments thereof lacking all or part of an immunoglobulin constant region, e.g., Ev. Fab. or F(ab')s and the like

"Altered immunoglobulin coding region" refers to a nucleic acid sequence encoding an altered antibody of the invention or a fragment thereof

"Reshaped human antibody" refers to an altered antibody in which minimally at least one CDR from a first human monoclonal donor antibody is substituted for a CDR in a second human acceptor antibody. Preferrably all six CDRs are replaced More preferrably an entire antigen combining region (e.g., Fv, Fab or F(ab')₂) from a first human donor monoclonal antibody is substituted for the corresponding region in a second human acceptor monoclonal antibody. Most preferrably the Fab region from a first human donor is operatively linked to the appropriate constant regions of a second human acceptor antibody to form a full length monoclonal antibody. The reshaped human monoclonal antibodies designated herein as Hu19A. Hu19B, Hu19C and Hu19D are defined as reshaped human antibodies comprising a light chain amino acid sequence selected from Sequences 19A, 19B, 19C and 19D of

chain amino acid sequence selected from Sequences 19A, 19B, 19C and 19D of Figure 3 and a heavy chain amino acid sequence selected from Sequences 19A, 19B, 19C and 19D of Figure 2, or functional partial sequences thereof.

"First immunoglobulin partner" refers to a nucleic acid sequence encoding a human framework or human immunoglobulin variable region in which the native (or naturally-occurring) CDR-encoding regions are replaced by the CDR-encoding regions of a donor human antibody. The human variable region can be an immunoglobulin heavy chain, a light chain (or both chains), an analog or functional fragments thereof. Such CDR regions, located within the variable region of antibodies (immunoglobulins) can be determined by known methods in the art. For example Kabat et al. (Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)) disclose rules for locating CDRs. In addition, computer programs are known which are useful for identifying CDR regions/structures.

"Second fusion partner" refers to another nucleotide sequence encoding a protein or peptide to which the first immunoglobulin partner is fused in frame or by

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means of an optional conventional linker sequence (i.e., operatively linked). Preferably the fusion partner is an immunoglobulin gene and when so, it is referred to as a "second immunoglobulin partner". The second immunoglobulin partner may include a nucleic acid sequence encoding the entire constant region for the same (i.e., homologous - the first and second altered antibodies are derived from the same source) or an additional (i.e., heterologous) antibody of interest. It may be an immunoglobulin heavy chain or light chain (or both chains as part of a single polypeptide). The second immunoglobulin partner is not limited to a particular immunoglobulin class or isotype. In addition, the second immunoglobulin partner may comprise part of an immunoglobulin constant region, such as found in a Fab, or F(ab)₂ (i.e., a discrete part of an appropriate human constant region or framework region). A second fusion partner may also comprise a sequence encoding an integral membrane protein exposed on the outer surface of a host cell, e.g., as part of a phage display library, or a sequence encoding a protein for analytical or diagnostic detection, e.g., horseradish peroxidase, β-galactosidase, etc.

The terms Fv, Fc, Fd, Fab, or F(ab')₂ are used with their standard meanings (see, e.g., Harlow et al., <u>Antibodies A Laboratory Manual</u>, Cold Spring Harbor Laboratory, (1988)).

As used herein, an "engineered antibody" describes a type of altered antibody, i.e., a full-length synthetic antibody (e.g., a chimeric, humanized, reshaped or immunologically edited human antibody as opposed to an antibody fragment) in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous parts from one or more donor antibodies which have specificity for the selected epitope. For example, such molecules may include antibodies characterized by a humanized heavy chain associated with an unmodified light chain (or chimeric light chain), or vice versa. Engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the acceptor antibody light and/or heavy variable domain framework regions in order to retain donor antibody binding specificity. These antibodies can comprise replacement of one or more CDRs (preferably all) from the acceptor antibody with CDRs from a donor antibody described herein.

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A "chimeric antibody" refers to a type of engineered antibody which contains naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody from a heterologous species.

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al., Proc. Nat'l. Acad. Sci. USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)).

An "immunologically edited antibody" refers to a type of engineered antibody in which changes are made in donor and/or acceptor sequences to edit regions in respect of cloning artifacts, germ line enhancements, etc. aimed at reducing the likelihood of an immunological response to the antibody on the part of a patient being treated with the edited antibody.

The term "donor antibody" refers to an antibody (monoclonal, or recombinant) which contributes the nucleic acid sequences of its variable regions. CDRs, or other functional fragments or analogs thereof to a first immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody. One donor antibody suitable for use in this invention is a Fab fragment of a human neutralizing monoclonal antibody designated as Fab Hu19. Fab Hu19 is defined as a having the variable light chain DNA and amino acid sequences Hu 19A as shown in Figures 2, 3, 4A and 4B.

The term "acceptor antibody" refers to an antibody (monoclonal, or recombinant) from a source genetically unrelated to the donor antibody, which contributes all (or any portion, but preferably all) of the nucleic acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. Preferably a human antibody is the acceptor antibody.

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"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate). CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include analogs of the naturally occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived.

By "sharing the antigen binding specificity or neutralizing ability" is meant, for example, that although Fab Hu19 may be characterized by a certain level of antigen affinity, a CDR encoded by a nucleic acid sequence of Fab Hu19 in an appropriate structural environment may have a lower, or higher affinity. It is expected that CDRs of Fab Hu19 in such environments will nevertheless recognize the same epitope(s) as does the intact Fab Hu19. A "functional fragment" is a partial heavy or light chain variable sequence (e.g., minor deletions at the amino or carboxy terminus of the immunoglobulin variable region) which retains the same antigen binding specificity and/or neutralizing ability as the antibody from which the fragment was derived.

An "analog" is an amino acid sequence modified by at least one amino acid, wherein said modification can be chemical or a substitution or a rearrangement of a few amino acids (i.e., no more than 10), which modification permits the amino acid sequence to retain the biological characteristics, e.g., antigen specificity and high affinity, of the unmodified sequence. For example, (silent) mutations can be constructed, via substitutions, when certain endonuclease restriction sites are created within or surrounding CDR-encoding regions.

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Analogs may also arise as allelic variations. An "allelic variation or modification" is an alteration in the nucleic acid sequence encoding the amino acid or peptide sequences of the invention. Such variations or modifications may be due to degeneracy in the genetic code or may be deliberately engineered to provide desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

The term "effector agents" refers to non-protein carrier molecules to which the altered antibodies, and/or natural or synthetic light or heavy chains of the donor antibody or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used in the diagnostic field, e.g., polystyrene or other plastic beads, polysaccharides, e.g., as used in the BIAcore (Pharmacia) system, or other non-protein substances useful in the medical field and safe for administration to humans and animals. Other effector agents may include a macrocycle, for chelating a heavy metal atom, or radioisotopes. Such effector agents may also be useful to increase the half-life of the altered antibodies, e.g., polyethylene glycol.

II. Combinatorial Cloning:

As mentioned above, a number of problems have hampered the direct application of the hybridoma technology of G. Kohler and C. Milstein (Nature 256: 495-497 (1975)) to the generation and isolation of human monoclonal antibodies. Among these are a lack of suitable fusion partner myeloma cell lines used to form hybridoma cell lines as well as the poor stability of such hybridomas even when formed. These shortcomings are further exacerbated in the case of RSV because of the paucity of viral specific B cells in the perpherial circulation. Therefore, the molecular biological approach of combinatorial cloning is preferred.

Combinatorial cloning is disclosed generally in PCT Publication No. WO90/14430. Simply stated, the goal of combinatorial cloning is to transfer to a population of bacterial cells the immunological genetic capacity of a human cell. tissue or organ. It is preferred to employ cells, tissues or organs which are immunocompetent. Particularly useful sources include, without limitation, spleen.

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thymus, lymph nodes, bone marrow, tonsil and perpherial blood lymphocytes. The cells may be optionally RSV stimulated *in vitro*, or selected from donors which are known to have produced an immune response or donors who are HIV+ but asymptomatic.

The genetic information isolated from the donor cells can be in the form of DNA or RNA and is conveniently amplified by Polymerase Chain Reaction (PCR) or similar techniques. When isolated as RNA the genetic information is preferably converted into cDNA by reverse transcription prior to amplification. The amplification can be generalized or more specifically tailored. For example, by a careful selection of PCR primer sequences, selective amplification of immunoglobulin genes or subsets within that class of genes can be achieved.

Once the component gene sequences are obtained, in this case the genes encoding the variable regions of the various heavy and light antibody chains, the light and heavy chain genes are associated in random combinations to form a random combinatorial library. Various recombinant DNA vector systems have been described to facilitate combinatorial cloning (see: PCT Publication No. WO90/14430 supra, Scott and Smith, Science 249:386-406 (1990) or U. S. Patent 5,223,409). Having generated the combinatorial library, the products can, after expression, be conveniently screened by biopanning with RSV F protein or, if necessary, by epitope blocked biopanning as described in more detail below.

Initially it is generally preferred to use Fab fragments of mAbs for combinatorial cloning and screening and then to convert the Fabs to full length mAbs after selection of the desired candidate molecules. However, single chain antibodies can also be used for cloning and screening.

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III. Antibody Fragments

The present invention contemplates the use of Fab fragments or F(ab')₂ fragments to derive full-length mAbs directed against the F protein of RSV. Although these fragments may be independently useful as protective and therapeutic agents *in vivo* against RSV-mediated conditions or *in vitro* as part of an RSV diagnostic, they are employed herein as a component of a reshaped human antibody.

A Fab tragment contains the entire light chain and amino terminal portion of the heavy chain; and an F(ab'), fragment is the tragment formed by two Fab fragments bound by additional disulfide bonds. RSV binding monoclonal antibodies provide sources of Fab tragments and F(ab'), fragments and can be obtained via combinatorial phage library (see, e.g., Winter et al., Ann. Rev. Immunol., 12:433-455 (1994) or Barbas et al. (Proc. Nat'l. Acad. Sci. (USA) 89, 10164-10168 (1992)) which are both hereby incorporated by reference in their entirety).

IV. Anti-RSV Antibody Amino Acid and Nucleotide Sequences of Interest

The Fab Hu19 or other antibodies described herein may contribute sequences, such as variable heavy and/or light chain peptide sequences, framework sequences, CDR sequences, functional fragments, and analogs thereof, and the nucleic acid sequences encoding them, useful in designing and obtaining various altered antibodies which are characterized by the antigen binding specificity of the donor antibody.

As one example, the present invention thus provides variable light chain and variable heavy chain sequences from the RSV human Fab Hu19A-D and sequences derived therefrom.

The nucleic acid sequences of this invention, or fragments thereof, encoding the variable light chain and heavy chain peptide sequences are also useful for mutagenic introduction of specific changes within the nucleic acid sequences encoding the CDRs or framework regions, and for incorporation of the resulting modified or fusion nucleic acid sequence into a plasmid for expression. For example, silent substitutions in the nucleotide sequence of the framework and CDR-encoding regions can be used to create restriction enzyme sites which would facilitate insertion of mutagenized CDR (and/or framework) regions. These CDR-encoding regions may be used in the construction of reshaped human antibodies of this invention.

Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which encode the variable heavy and light chain amino acid sequences, and CDR sequences of the invention as well as functional

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fragments and analogs thereof which share the antigen specificity of the donor antibody. The isolated nucleic acid sequences of this invention, or fragments thereof, encoding the variable chain peptide sequences or CDRs can be used to produce altered antibodies, e.g., chimeric or humanized antibodies, or other engineered antibodies of this invention when operatively combined with a second immunoglobulin partner.

It should be noted that in addition to isolated nucleic acid sequences encoding portions of the altered antibody and antibodies described herein, other such nucleic acid sequences are encompassed by the present invention, such as those complementary to the native CDR-encoding sequences or complementary to the modified human framework regions surrounding the CDR-encoding regions. Such sequences include all nucleic acid sequences which by virtue of the redundancy of the genetic code are capable of encoding the same amino acid sequence as given in Figures 2 and 3. Other useful DNA sequences encompassed by this invention include those sequences which hybridize under stringent hybridization conditions (See: T. Maniatis et al., Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389) to the DNA sequences encoding the antibodies of Figures 2 and 3 and which retain the antigen binding properties of those antibodies. An example of one such stringent hybridization condition is hybridization at 4XSSC at 65°C, followed by a washing in 0.1XSSC at 65°C for an hour. Alternatively an exemplary stringent hybridization condition is in 50% formamide, 4XSSC at 42°C. Preferably, these hybridizing DNA sequences are at least about 18 nucleotides in length, i.e., about the size of a CDR.

V. Altered Immunoglobulin Coding Regions and Altered Antibodies

Altered immunoglobulin coding regions encode altered antibodies which
include engineered antibodies such as chimeric antibodies, humanized, reshaped and
immunologically edited human antibodies. A desired altered immunoglobulin
coding region contains CDR-encoding regions in the form of Fab regions that
encode peptides having the antigen specificity of an RSV antibody, preferably a high

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affinity antibody such as provided by the present invention, inserted into an acceptor immunoglobulin partner.

When the acceptor is an immunoglobulin partner, as defined above, it includes a sequence encoding a second antibody region of interest, for example an Fe region. Immunoglobulin partners may also include sequences encoding another immunoglobulin to which the light or heavy chain constant region is fused in frame or by means of a linker sequence. Engineered antibodies directed against functional fragments or analogs of RSV may be designed to elicit enhanced binding with the same antibody.

The immunoglobulin partner may also be associated with effector agents as defined above, including non-protein carrier molecules, to which the immunoglobulin partner may be operatively linked by conventional means.

Fusion or linkage between the immunoglobulin partners, e.g., antibody sequences, and the effector agent may be by any suitable means, e.g., by conventional covalent or ionic bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, and the like. Such techniques are known in the art and readily described in conventional chemistry and biochemistry texts.

Additionally, conventional linker sequences which simply provide for a desired amount of space between the second immunoglobulin partner and the effector agent may also be constructed into the altered immunoglobulin coding region. The design of such linkers is well known to those of skill in the art.

In addition, signal sequences for the molecules of the invention may be modified to enhance expression. For example the reshaped human antibody having the signal sequence and CDRs derived from the Fab Hu19 heavy chain sequence, may have the original signal peptide replaced with another signal sequence such as the Campath leader sequence (Page, M. J. et al., BioTechnology 9:64-68(1991)).

An exemplary altered antibody, a reshaped human antibody, contains a variable heavy and the entire light chain peptide or protein sequence having the antigen specificity of Fab Hu19, fused to the constant heavy regions C_{H-1} - C_{H-3} derived from a second human antibody.

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In still a further embodiment, the engineered antibody of the invention may have attached to it an additional agent. For example, the procedure of recombinant DNA technology may be used to produce an engineered antibody of the invention in which the Fc fragment or CH2 CH3 domain of a complete antibody molecule has been replaced by an enzyme or other detectable molecule (i.e., a polypeptide effector or reporter molecule).

Another desirable protein of this invention may comprise a complete antibody molecule, having full length heavy and light chains, or any discrete fragment thereof, such as the Fab or F(ab')₂ fragments, a heavy chain dimer, or any minimal recombinant fragments thereof such as an F_v or a single-chain antibody (SCA) or any other molecule with the same specificity as the selected donor Fab Hu19. Such protein may be used in the form of an altered antibody, or may be used in its unfused form.

Whenever the immunoglobulin partner is derived from an antibody different from the donor antibody, e.g., any isotype or class of immunoglobulin framework or constant regions, an engineered antibody results. Engineered antibodies can comprise immunoglobulin (Ig) constant regions and variable framework regions from one source, e.g., the acceptor antibody, and one or more (preferably all) CDRs from the donor antibody, e.g., the anti-RSV antibody described herein. In addition, alterations, e.g., deletions, substitutions, or additions, of the acceptor mAb light and/or heavy variable domain framework region at the nucleic acid or amino acid levels, or the donor CDR regions may be made in order to retain donor antibody antigen binding specificity or to reduce potential immunogenicity. In the present invention, a preferred mutation is the alteration of the consensus N-linked glycosylation site in CDR2 of the Hu19A and Hu19B heavy chain, as exemplified in the heavy chains of Hu19C and Hu19D (Fig. 2) (SEQ ID NO'S 7 and 8).

Such engineered antibodies are designed to employ one (or both) of the variable heavy and/or light chains of the RSV mAb (optionally modified as described) or one or more of the below-identified heavy or light chain CDRs. The engineered antibodies of the invention are neutralizing, i.e., they desirably inhibit virus growth *in vitro* and *in vivo* in animal models of RSV infection.

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Such engineered antibodies may include a reshaped human antibody containing the human heavy and light chain constant regions fused to the RSV antibody functional fragments. A suitable human (or other animal) acceptor antibody may be one selected from a conventional database, e.g., the KABAT database. Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody.

Desirably the heterologous framework and constant regions are selected from human immunoglobulin classes and isotypes, such as IgG (subtypes 1 through 4), IgM. IgA and IgE. The Fc domains are not limited to native sequences, but include mutant variants known in the art that alter function. For example, mutations have been described in the Fc domains of certain IgG antibodies that reduce Fc-mediated complement and Fc receptor binding (see, e.g., A. R. Duncan et al., Nature 332:563-564 (1988); A. R. Duncan and G. Winter, Nature 332:738-740 (1988); M.-L. Alegre et al., J. Immunol. 148:3461-3468 (1992); M.-H. Tao et al., J. Exp. Med. 178:661-667 (1993); V. Xu et al. J. Biol. Chem., 269:3469-2374 (1994)), alter clearance rate (J.-K. Kim et al., Eur. J. Immunol. 24:542-548 (1994), and reduce structural heterogeneity (S. Angal et al., Mol. Immunol. 30:105-108 (1993)). Also, other modifications are possible such as oligomerization of the antibody by addition of the tailpiece segment of IgM and other mutations (R. I. F. Smith and S. L. Morrison. Biotechnology 12:683-688 (1994); R. I. F. Smith et al., J. Immunol., 154: 2226-2236 (1995)) or addition of the tailpiece segment of IgA (I. Kariv et al., J. Immunol. 157: 29-38 (1996). However, the acceptor antibody need not comprise only human immunoglobulin protein sequences. For instance a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to

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a DNA sequence encoding a non-immunoglobulin amino acid sequence such as a polypeptide effector or reporter molecule.

The altered antibody thus preferably has the structure of a natural human antibody or a fragment thereof, and possesses the combination of properties required for effective therapeutic use, e.g., treatment of RSV mediated diseases in man, or for diagnostic uses.

It will be understood by those skilled in the art that an altered antibody may be further modified by changes in variable domain amino acids without necessarily affecting the specificity and high affinity of the donor antibody (i.e., an analog). It is anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the variable domain frameworks or CDRs or both. Particularly preferred is the immunological editing of such reconstructed sequences as illustrated in the examples herein.

In addition, the variable or constant region may be altered to enhance or decrease selective properties of the molecules of the instant invention, as described above. For example, dimerization, binding to Fc receptors, or the ability to bind and activate complement (see, e.g., Angal et al., Mol. Immunol, 30:105-108 (1993), Xu et al., J. Biol. Chem, 269:3469-3474 (1994), Winter et al., EP 307,434-B).

Such antibodies are useful in the prevention and treatment of RSV mediated disorders, as discussed below.

VI. Production of Altered antibodies and Engineered Antibodies

The resulting reshaped human antibodies of this invention can be expressed in recombinant host cells, e.g., COS, CHO or myeloma cells. A conventional expression vector or recombinant plasmid is produced by placing these coding sequences for the altered antibody in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g., CMV promoter, and signal sequences, which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antibody light or heavy

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chain. Preferably this second expression vector is identical to the first except insofar as the coding sequences and selectable markers are concerned, so to ensure as far as possible that each polypeptide chain is functionally expressed. Alternatively, the heavy and light chain coding sequences for the altered antibody may reside on a single vector.

A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention. The production of the antibody which includes the association of both the recombinant heavy chain and light chain is measured in the culture by an appropriate assay, such as ELISA or RIA. Similar conventional techniques may be employed to construct other altered antibodies and molecules of this invention.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the conventional pUC series of cloning vectors, may be used. One vector used is pUC19, which is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or Pharmacia (Uppsala, Sweden). Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

Similarly, the vectors employed for expression of the engineered antibodies according to this invention may be selected by one of skill in the art from any conventional vectors. Preferred vectors include for example plasmids pCD or pCN. The vectors also contain selected regulatory sequences (such as CMV promoters) which direct the replication and expression of heterologous DNA sequences in selected host cells. These vectors contain the above described DNA sequences which code for the engineered antibody or altered immunoglobulin coding region.

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In addition, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the engineered antibodies or altered immunoglobulin molecules thereof. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of *E. coli* are used for replication of the cloning vectors and other steps in the construction of altered antibodies of this invention.

Suitable host cells or cell lines for the expression of the engineered antibody or altered antibody of the invention are preferably mammalian cells such as CHO, COS, a fibroblast cell (e.g., 3T3), and myeloid cells, and more preferably a CHO or a myeloid cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook et al., cited above.

Bacterial cells may prove useful as host cells suitable for the expression of the recombinant Fabs of the present invention (see, e.g., Plückthun, A., <u>Immunol</u>.

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Rev., 130:151-188 (1992)) The tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form does not pose as great a concern as Fabs are not normally glycosylated and can be engineered for exported expression thereby reducing the high concentration that facilitates misfolding. Nevertheless, any recombinant Fab produced in a bacterial cell would have to be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced and exported in a properly folded form, that bacterial cell would be a desirable host. For example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis, Streptomyces*, other bacilli and the like may also be

Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be employed in this method.

Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. *Drosophila* and *Lepidoptera* and viral expression systems. See, e.g. Miller et al., Genetic Engineering, 8:277-298.

Plenum Press (1986) and references cited therein.

The general methods by which the vectors of the invention may be constructed, the transfection methods required to produce the host cells of the invention, and culture methods necessary to produce the altered antibody of the invention from such host cell are all conventional techniques. Likewise, once produced, the altered antibodies of the invention may be purified from the cell culture contents according to standard procedures of the art, including ammonium surfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention.

Yet another method of expression of resphaped antibodies may utilize expression in a transgenic animal, such as described in U. S. Patent No. 4.873.316. This relates to an expression system using the animal's casein promoter which when transgenically incorporated into a mammal permits the female to produce the desired recombinant protein in its milk.

Once expressed by the desired method, the engineered antibody is then examined for *in vitro* activity by use of an appropriate assay. Presently conventional

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ELISA assay formats are employed to assess qualitative and quantitative binding of the altered antibody to RSV. Additionally, other *in vitro* assays and *in vivo* animal models may also be used to verify neutralizing efficacy prior to subsequent human clinical studies performed to evaluate the persistence of the altered antibody in the body despite the usual clearance mechanisms.

VII. Therapeutic/Prophylactic Uses

This invention also relates to a method of treating humans experiencing RSV-related symptoms which comprises administering an effective dose of antibodies including one or more of the altered antibodies described herein or fragments thereof.

The therapeutic response induced by the use of the molecules of this invention is produced by the binding to RSV and thus subsequently blocking RSV propagation. Thus, the molecules of the present invention, when in preparations and formulations appropriate for therapeutic use, are highly desirable for those persons experiencing RSV infection. For example, longer treatments may be desirable when treating seasonal episodes or the like. The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient.

The altered antibodies, antibodies and fragments thereof of this invention may also be used alone or in conjunction with other antibodies, particularly human or humanized mAbs reactive with other epitopes on the F protein or other RSV target antigens as prophylatic agents.

The mode of administration of the therapeutic and prophylatic agents of the invention may be any suitable route which delivers the agent to the host. The altered antibodies, antibodies, engineered antibodies, and fragments thereof, and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly, intravenously, or intranasally.

Therapeutic and prophylacticagents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the altered antibody

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of the invention as an active ingredient in a pharmaceutically acceptable carrier. An aqueous suspension or solution containing the antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the engineered antibody of the invention or a cocktail thereof dissolved in an pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.4% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antibody of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 80 mg or more preferably, about 5 mg to about 75 mg of an engineered antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 75 and preferably 5 to about 50 mg/ml of an engineered antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania.

It is preferred that the therapeutic and prophylactic agents of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. To effectively treat an inflammatory disorder in a human or other animal.

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one dose of approximately 0.1 mg to approximately 20 mg per 70 kg body weight of a protein or an antibody of this invention should be administered parenterally, preferably i.v. or i.m. (intramuscularly). Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician.

The altered antibodies and engineered antibodies of this invention may also be used in diagnostic regimens, such as for the determination of RSV mediated disorders or tracking progress of treatment of such disorders. As diagnostic reagents, these altered antibodies may be conventionally labeled for use in ELISAs and other conventional assay formats for the measurement of RSV levels in serum, plasma or other appropriate tissue, or the release by human cells in culture. The nature of the assay in which the altered antibodies are used are conventional and do not limit this disclosure.

The antibodies, altered antibodies or fragments thereof described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

The following examples illustrate various aspects of this invention including the construction of exemplary engineered antibodies and expression thereof in suitable vectors and host cells, and are not to be construed as limiting the scope of this invention. All amino acids are identified by conventional three letter or single letter codes. All necessary restriction enzymes, plasmids, and other reagents and materials were obtained from commercial sources unless otherwise indicated. All general cloning ligation and other recombinant DNA methodology were as performed in T. Maniatis et al., cited above, or the second edition thereof (1989), eds. Sambrook et al., by the same publisher ("Sambrook et al.").

Example A

Conversion of Hu19 Fab to mAb Version A: Direct Cloning

For expression in mammalian cells, the heavy chain variable region and the light chain variable and constant regions from the Fab clone 19 plasmid(C. Barbas III et al., Proc. Nat'l. Acad. Sci. (USA) 89, 10164-10168 (1992) and PCT application

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Publication No. WO 94/06448, Application No. PCT/US93/08786 Cell Line Clone 19 referenced therein as ATCC Accession No. 69072) herein designated Hu19 Fab, were cloned into derivatives of plasmid PCDN (Nambi, A. et.al., Molecular and Cellular Biochemistry 131:75-86 (1994), in which the expression of the antibody chain is driven by the CMV promoter. Plasmid PCD-HC68B is used for cloning and expressing full length heavy chains and plasmid PCN-HuLC, for cloning and expressing full length light chains (Figure 1 shows the strategy for cloning of version A of the Hu19 mAb).

In the initial constructs, the changes in the sequence at the amino terminus. introduced by the PCR primers used for cloning, were not altered. For the heavy chain, the variable region was extracted from the Hu19 Fab plasmid (C. Barbas III et al., Proc. Nat'l. Acad. Sci. (USA) 89, 10164-10168 (1992)) as an Xho1-Bsp1201 fragment and introduced into the same sites in PCD-HC68B. The Xho1 site was introduced at the amino terminus by the PCR primer and, when cloned into PCD-HC68B at the same site is preceded in frame by the Campath leader sequence (Page, J.M. et al., Biotechnology 9:64-68 (1991). The Bsp120I site is a naturally occurring. highly conserved sequence at the beginning of the CH1 domain, and when cloned into PCD-HC68B at the same site is in frame with the remaining sequence for the CH1 through CH3 regions of human IgG1 (Figure 1). In the resulting construct, ''119AHcpcd, the amino acids immediately following the Campath leader are EVQLLEE (Fig. 2 SEQ ID NO 5, AMINO ACIDS 20 - 26), where the residues LE are encoded by the nucleotide sequence for the Xho1 cloning site. The complete nucleotide sequence for the plasmid Hu19AHcpcd is shown in Fig. 4A (SEQ ID NO) 14).

Of note, sequence analysis revealed base differences from the published sequence (C. Barbas III et al., Proc. Nat'l. Acad. Sci. (USA) 89, 10164-10168 (1992), PCT publication WO94/06448) within the heavy chain region from the Hu19 Fab plasmid. The changes encode amino acid differences at positions 15 and 16 (14 and 15 according to consensus numbering of Kabat et al (Sequences of Proteins of Immunological Interest, fifth edition, NIH Publication No. 91-3242, 1991):

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PG in the Hu19AHcpcd vector versus LA in the published sequence (see Fig 2 of this application and Fig. 4 of WO94/06448)). This discrepancy must represent an error in the original published sequence. PG at these positions is the consensus sequence in the closest homologues among published human antibodies (Kabat et al., Sequences of Proteins of Immunological Interest, fifth edition, NIH Publication No. 91-3242, 1991) and in the likely germline parent sequence (see below, version B). In addition, sequences derived from 3 independent clonings initiated with the Hu19 Fab plasmid all encoded PG at these positions.

For the light chain, the variable and constant regions of the Hul9 Fab plasmid were cloned as a Sac1/Xba1 fragment into the same sites in the pCN-HuLcvector. Both restriction sites correspond to restriction sites introduced by the primers used in the PCR amplification. The Sac1 site is introduced at the amino terminus by the PCR primer and, when cloned into pCN-HuLC at the same site, is preceded in frame by the Campath leader sequence (Page, J.M. et al., Biotechnology 9:64-68 (1991). The first 2 amino acids of the mature light chain are therefore deleted. In the resulting construct, Hu19ALcpcn, the first 2 amino acids immediately following the leader are EL (Fig. 3, part A), where the residues EL are encoded by the nucleotide sequence for the Sac1 cloning site. The PCR primer used at the carboxy terminus of the constant region introduces a nucleotide substitution which changes the amino acid at position 202 of the mature light chain, from a serine to a leucine (Fig 3, part B). The Xba1 restriction site, introduced by the same PCR primer, lies outside the coding region and has no effect on the final amino acid sequence of the mature light chain. The complete nucleotide sequence of the plasmid Hull9Apen is shown in Fig. 4B.

As for the heavy chain above, there was a sequence discrepancy for the light chain between the published sequence (C. Barbas III et al., Proc. Nat'l. Acad. Sci. (USA) 89: 10164-10168 (1992), PCT publication WO94/06448) and the sequence obtained in the Hu19ALcpcn vector. A single base change resulted in glycine in Hu19ALcpcn in place of glutamic acid at position 97 (also consensus position 97 in Kabat et al (Sequences of Proteins of Immunological Interest, fifth edition, NIH Publication No. 91-3242, 1991)) in framework 4 (see Fig. 3 of this application and

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Fig. 4 of WO94/06448). Glycine, but not glutamic acid, is encoded at this position in a human J germline J mini-gene and glutamic acid was not observed among a large collection of human antibody sequences (Kabat et al., "Sequences of Proteins of Immunological Interest", fifth edition, NIH Publication No. 91-3242, 1991). Also as for the heavy chain, the glycine encoding sequence was observed for 3 separate clonings from the original Fab 19 vector (Barbas et al., Proc. Nat'l. Acad. Sci. (USA) 89, 10164-10168 (1992), PCT publication WO94/06448). These results demonstrate that the originally published sequence for Fab 19 light chain is in error.

The Hu19AHcpcd and Hu19ALcpcn set of vectors were used to produce antibody Hu19A in COS cells and in CHO cells.

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Example B

Version B: Cloning Of The Edited Fab Hu19 Heavy and Light Chains

In cloning the variable region of the Fab 19 heavy chain, non-consensus amino acid changes relative to the predicted germline sequence were introduced at the amino terminus by the PCR primer (C. Barbas III et al., Proc. Nat'l. Acad. Sci. (USA) 89, 10164-10168 (1992)). To determine the likely amino terminus of the heavy chain, the peptide sequence of the variable region of the Fab 19 heavy chain was aligned with all known human germline heavy chain sequences. Based on the results of this alignment, the germline amino terminus is predicted to be either QVQLVE or EVQLVE rather than the sequence EVQLLEE present in version A. To correct the N-terminus, the original Fab clone 19 heavy chain peptide was aligned with human heavy chain sequences previously cloned at SmithKline Beecham. A clone designated 97B27, which was obtained via PCR amplification from the beginning of its leader sequence, had the acceptable N-terminus of QVQLVE and was used to replace this region in the Fab19 heavy chain. Specifically, the Fab19 heavy chain in the Hu19 Fab plasmid was PCR amplified using a constant region primer which spanned the naturally occurring Bsp120 I site at the beginning of CH1, and a variable region primer which created a PvuII site 20 (corresponding to the site naturally occurring in clone 97B27) at amino acids 3 and 4 of the mature protein. This primer also introduced changes in the coding sequence at the amino terminus of the Fab19 heavy chain, coding for the amino acid sequence of QLVE for amino acids 3-6 instead of QLLEE, as in the version A construct. The PCR fragment was cut with restriction enzymes PvuII and Bsp 120I, and, through a 25 series of cloning steps, was combined with 97B27 at its PvuII site. The resulting clone, designated Hu19BHcpcd, contained the leader and first 3 amino acids of the variable region of clone 97B27 and coded for the consensus sequence QVQLVE at its amino terminus (Fig. 2). The nucleotide sequence of Hu19BHcpcd is shown in Fig. 4C (SEQ ID NO: 20) for the region encoding the heavy chain. Sequences 30 differing from Hu19AHcpcd are bolded.

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In cloning the variable region of the Fab clone 19 light chain, changes were introduced at the amino terminus for cloning purposes, by the PCR primer, such that the first 4 amino acids of the Fab19 light chain are EIEL. To determine the likely ammo terminus of the light chain, the peptide sequence of the variable region of the Fab19 light chain was aligned with all known human germline kappa chain sequences. Based on the results of this alignment, the germline amino terminus is predicted to be DIQM. To convert the amino terminus of Fab19 Le to the predicted germline sequence, Fab19 light chain was aligned with human kappa chain sequences previously cloned at SB. A Clone designated AG1-37, which is the kappa chain obtained from cell line AG1-37 obtained by PCR amplification from the middle of its leader sequence, had the desired N-terminus and was used to introduce the corrections into the Fab19 light chain. The N-terminal portion of the leader sequence was provided by the expression vector and was the consensus sequence for this family of leader regions. For this construct, the light chain coding region was excised from the Hu19 Fab vector (Fig. 1) as a *HinfVXba* I fragment. *Hinf* 1 recognizes a site which spans amino acids 18 an 19 of the mature protein and is also present in clone AG1-37. Through a series of cloning steps, the Hinf1/Xba1 fragment of the Fab19 light chain was ligated to the *Hinf*1 site in clone AG1-37. The final construct consisted of the leader and first 18 amino acids of the AG1-37 variable region linked to the variable and constant regions of the Fab 19 light chain. beginning at amino acid 19 of the V-region. The resulting clone, designated Hull9BLcpcn, is altered only in the region encoding the first four amino acids of the variable region, coding for the consensus sequence DIQM (SEQ ID NO: 11, AMINO) ACIDS 21 - 24) instead of EIEL present in version A (Fig. 3A). The nucleotide sequence for plasmid Hu19BLcpcn is shown in Fig. 4D (SEQ ID NO: 22) for the region encoding the light chain. Sequences differing from Hu19ALcpcn are bolded.

The vector set of Hu19BHcpcd and Hu19BLcpcn was used to produce antibody Hu19B in COS cells and in CHO cells.

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Example C

Versions C & D: Mutation Of CDR2 Of Hu19B Heavy Chain To Eliminate a Glycosylation Site

An N-linked glycosylation site is encoded within the CDR2 loop of the heavy chain. This glycosylation adds the potential for heterogeneity in the mAb produced in eucaryotic cells and for interference in binding antigen. To eliminate this glycosylation site, mutations were introduced separately at two different residues via PCR overlap technology. For the first mutation the serine at position 61 of the mature Hu19B heavy chain was substituted with alanine, to create Hu19C heavy chain. For the second substitution, the asparagine at position 59 was changed to glutamine, to create Hu19D heavy chain.

	SITGGSNGINY <u>A</u> DSVKR	S61A Subst	titution	(SEQ ID	NO: 1)
15	SITGGSNGI NYS DSVKR	Original	HuB CDR2	SEQ ID) NO: 2)
	SITGGSNGI O YSDSVKR	N590 Subs	titution	(SEO ID	NO: 3)

Specifically, the mutations were introduced via the PCR overlap technique using one set of primers encoding the mutation and a second set of primers annealing to sequences within the CMV promoter and the CH2 constant region in plasmid Hu19Bpcd, as the outside 5' and 3' primers, respectfully. The final PCR product was digested with restriction enzymes, EcoR1 and Bsp120I, and cloned into the Hu19BHcpcd vector at the same sites to create Hu19CHcpcd (Ser to Ala mutation) and Hu19DLcpcd (Asp to Gln mutation) (Fig. 2). The final constructs were sequenced to verify that the mutations were present. The nucleotide sequences of the heavy chain regions in Hu19CHcpcd and Hu19DHcpcd are shown in Figs. 4E and 4F (SEQ ID NO'S 25 AND 27). Differences from Hu19Hcpcd are bolded.

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Example D

Version C: Cloning Of The Edited Constant Region

In the original cloning the of the Fab19 light chain, a change was purposely introduced at the caboxy terminus by the PCR primer to eliminate a naturally occurring Sac1 site (Barbas et al. <u>supra</u>). Consequently, the amino acid at position 202 of the Fab19 light chain was changed from a serine to a leucine. This change was corrected as follows. Plasmid Hu19BLepen was cut with EcoR1 and Bbs1, a naturally occurring restriction site near the amino terminus of human kappa constant region and a 405 bp fragment, containing the nucleotide sequence coding for the leader, variable region, and first 5 amino acids of the kappa constant region, was isolated. Plasmid Levector4, a puc18 derivative containing a normal human kappa constant region with a XbaI site just distal to the coding region, was cut with Bbs1 and Xba1 and a 321 bp fragment containing the nucleotide sequence coding for the entire kappa constant region beginning at amino acid 6 was isolated. This fragment contains the naturally occurring Sac1 site near the end of the carboxy terminus and codes for a serine at position 202. Plasmid Hu19BLepen was also cut with EcoR1and Xba and a 4947 bp fragment, containing the remainder of the vector sequence from plasmid Hu19BLepen, was isolated. The three fragments were ligated together to create Hu19CLepcn. The amino acid sequence of the Hu19C light chain is shown in Figs. 3A and 3B (SEQ ID NO'S 11 and 12) and the nucleotide sequence of the light chain region is shown in Fig 4G (SEQ ID NO: 29). Differences from Hu19ALcpcn are bolded. The vector Hu19CLcpcn, was used with Hu19CHcpcd or Hu19DHcpcd to produce antibody Hu19C and Hu19D, respectively, in COS cells and in CHO cells.

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Example E

Production of Hu19 Mabs in mammalian cells:

For initial characterization, the mAb constructs for each version, Hu19A heavy and light chain, Hu19B heavy and light chain, Hu19C heavy and light chain, and Hu19D heavy with Hu19C light chain, were expressed in COS cells essentially as described in Current Protocols in Molecular Biology (edited by F. M. Ausubel et

al. 1988. John Wiley & Sons, vol. 1, section 9.1). On day 1 after the transfection, the culture growth medium was replaced with a serum-free medium which was changed on day 3. The serum-free medium was a proprietary formulation but satisfactory results are obtained using DMEM supplemented with ITSTM Premix (insulin, transferrin, selenium mixture - Collaborative Research, Bedford, MA) and 1 mg/ml BSA. The mAb was prepared from the day 3 + day 5 conditioned medium by standard protein A affinity chromatography methods (e.g., as described in Protocols in Molecular Biology) using, for example, Prosep A affinity resin (Bioprocessing Ltd., UK).

To produce larger quantities of the Hu19 mAb (100-200 mgs), the vectors were introduced into a proprietary CHO cell system. However, similar results will be obtained using dhfr⁻ CHO cells as previously described (P. Hensley et al., J. Biological Chemistry 269:23949-23958 (1994)). Briefly, a total of 30ug of linearized plasmid DNA (15ug each of the A, B, C or D/C set of heavy chain and light chain vectors) was electroporated into 1x10⁷ cells. The cells were initially selected in nucleoside-free medium in 96 well plates. After three to four weeks, media from growth positive wells was screened for human immunoglobulin using an ELISA assay. The highest expressing colonies were expanded and selected in increasing concentrations of methotrexate for amplification of the transfected vectors. The antibody was purified from conditioned medium by standard procedures using protein A affinity chromatography (Protein A sepharose, Pharmacia) followed by size exclusion chromatography (Superdex 200, Pharmacia).

The concentration and the antigen binding activity of the eluted antibody are measured by ELISA. The antibody containing fractions are pooled and further purified by size exclusion chromatography. As expected for any such antibody, by SDS-PAGE, the predominant protein product migrated at approximately 150 kDa under non-reducing conditions and as 2 bands of 50 and 25 kDa under reducing conditions. For antibody produced in CHO cells, the purity was > 90%, as judged by SDS-PAGE, and the concentration was accurately determined by amino acid analysis.

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Example F

<u>Preparation of Fab from Hu19B mAb: Samples with and without glycosylation</u> in heavy chain CDR2

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Purification of MAbs

Each mAb was purified using an essentially similar purification procedure that is detailed here for mAb 19B. Conditioned media (2L) from a 6 day culture was harvested, sterile filtered and applied to a 2.5 X 5.1cm Protein A (Pharmacia, fast flow) equilibrated in 20mM sodium phosphate, 150mM sodium chloride, pH 7 (PBS) at a linear flow rate of 98cm/h. The column was washed with equilibration buffer and eluted with 100mM glycine pH 2.5. Elution fractions containing the mAb were immediately adjusted to pH 5.0 with 1M sodium hydroxide and applied at a concentration of 4.2 mg/mL to a Superdex 200 size exclusion column (2.6 x 70 cm) equilibrated in 20 mM sodium phosphate buffer containing 150 mM NaCl, pH7.0. Monomeric mAb that was retained by the column at an apparent molecular weight of around 150 kDa was pooled and concentrated by ultrafiltration to 5mg/mL, and stored at 4°C after sterile filtration.

20 Electrophoretic analysis of MAb19B and MAb 19C

By reduced SDS-PAGE, mAb 19B resolved as 2 major bands at 52 kDa and 28kDa corresponding to the heavy and light chains of IgG respectively, with an additional band at 59 kDa representing about 7% of the total protein (Fig. 5). LC/mass spectrometry analysis of the two heavy chains following excision from an SDS-PAGE and proteolytic digestion (see below). confirmed that the 59 kDa species

represented an additional glycoform of mAb 19B that contained carbohydrate at the predicted V_H glycosylation site. In contrast, reduced SDS-PAGE analysis of mAb 19C (Fig. 5), in which this V_H glycosylation site is removed, showed that this mAb contains only the lower molecular weight (52 kDa) heavy chain species, as expected.

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Carbohydrate Analysis of mAb 19B

The Hu19B construct contains an additional consensus sequence for N-linked glycosylation in the variable region of the heavy chain, -Asn⁵⁹-Tyr-Ser-, in addition to the normal glycosylation site in the C_H2 domain of the heavy chain, -Asn²⁹⁹-Ser
Thr-. Analysis of both heavy chain bands by liquid chromatography, electrospray mass spectrometry (LC-ELMS) following reduction, alkylation, and tryptic digestion revealed that the 59 kDa band contains a variant that is glycosylated at Asn⁵⁹ in addition to being glycosylated at Asn²⁹⁹. The carbohydrate at Asn⁵⁹ is predominantly biantennary, core fucosylated carbohydrates having two sialic acid residues. This is a common carbohydrate structure found in CHO-expressed glycoproteins (such as sCR-1 and sCD4), but it differs from the carbohydrate found at the Asn299 site which lacks sialic acid altogether.

Purification of mAb 19B Glycovariant

- 15 Mab 19B (2 mg) was dialyzed against 20 mM Tris, pH 8.5 and applied to a 0.5 x 5cm Mono Q column (Pharmacia) equilibrated in the same buffer at a linear flow rate of 300cm/h. The column was washed with equilibration buffer and eluted with a 20 column volume gradient from 0 mM to 50 mM NaCl in the same buffer (Fig.
- 6). Fractions containing the glycovariant, as determined by SDS-PAGE, were pooled, dialyzed against PBS, sterile filtered and stored at 4°C.

Preparation of Fabs by Proteolytic Digestion

mAb19B (48mg) was removed and the pH adjusted to 7.0 with dilute sodium hydroxide. 2.5ml of 100mM sodium phosphate buffer containing 10mM EDTA, pH 7.3; 1.3ml of 100mM cysteine in 10mM sodium phosphate buffer containing 1mM EDTA; and 20ul of crystalline papain (Boehringer, 10mg/ml) were added. The sample was incubated at 37°C for 20h and the digest applied to a 1.5 x 2.6cm Protein G column equilibrated in PBS at a linear flow rate of 67cm/h. The column was washed with PBS and the nonbound fraction containing the Fab was collected and concentrated to 5ml in an Amicon ultrafiltration cell fitted with a 10,000 molecular weight cut-off membrane and applied to a 2.6 X 70cm Superdex 200 (Pharmacia) size exclusion column equilibrated in PBS at a linear flow rate of 23cm/h. FAb (total yield, 12mg) eluted as a monomer on the size exclusion column and analysis by non-reduced SDS-PAGE revealed a major band at 45kDa and the glycoform at 47kDa.

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Separation of Fab Glycovariant

The mixture of glycosylated and unglycosylated Fab from cleaved mAb 19B was dialyzed against 20 mM sodium acetate, pH 4.5 and applied (4mg) to a 0.5 X 5cm Mono S column (Pharmacia) at 300cm/h equilibrated with 20mM sodium acetate buffer, pH 4.5. The column was then washed with equilibration buffer and eluted isocratically with the equilibration buffer containing 100 mM NaCl. Glycosylated Fab eluted after 5 column volumes whereas the unglycosylated FAb was retained longer, eluting after 6 column volumes. Fractions that contained only glycosylated Fab, as judged by SDS-PAGE, were pooled, diluted 1:1 with starting buffer and reapplied to a 0.16 X 5cm Mono S column at 300cm/h. The Fab was once again eluted with 100 mM NaCl and fractions most enriched for glycosylated Fab were pooled, dialyzed against PBS pH 7.0, and sterile filtered. By SDS-PAGE analysis this fraction was enriched >90% with the glycosylated species (Fig. 7). The process yielded 3.3 mg of unglycosylated Fab and 0.16 mg of glycosylated Fab, respectively.

Example G

Binding of Hu19 mAb and Fab clone 19 proteins to recombinant F protein

Binding of the various antibody constructs to recombinant F protein was measured in a standard solid phase ELISA. Antigen diluted in PBS pH 7.0 was adsorbed onto polystyrene round-bottom microplates (Dynatech, Immunolon II) for 18 hours. Wells were then aspirated and blocked with 0.5% boiled casein (BC) in PBS containing 1% Tween 20 (PBS/0.05% BC) for 2 hours. Antibodies (50 µl/well) were diluted to varying concentrations in PBS/0.5% BC containing 0.025% Tween 20 and incubated in antigen coated wells for one hour. Plates were washed three times with PBS containing 0.05% Tween 20, using a Titertek 320 microplate washer, followed by addition of HRP-labelled protein A/G (50 µl) diluted 1:5000. After washing three times, TMBlue substrate (TSI, #TM102) was added and plates were incubated an additional 15 minutes. The reaction was stopped by addition of 1 NH₂SO₄ and absorbance read at 450 nm using a Biotek ELISA reader.

The antigen binding epitope of Fab19 and mAb construct 19B were examined in a competition ELISA. The test antibody construct was mixed with increasing concentrations of RSMU19 or B4 and added to F protein-coated wells. The epitope regions recognized by mAbs RSMU19 and B4 have been previously described in Arbiza et al., J. Gen'l Virol. 73:2225-34 (1992). The concentration of Fab19 or mAb 19B used in competition studies was determined previously to give 90% maximal binding to F antigen. Binding of Fab19 or mAb 19B in the presence of other mAbs was detected using HRP-labelled goat anti-human IgG. The reaction was developed as stated above.

Fab19 and amAb constructs 19A or 19B, demonstrated equivalent binding to rF protein based on molar concentrations. Binding of Fab19 or mAb 19B to rF (recombinant F) protein was inhibited by mAb B4 but not by RSMU19 indicating that the epitope region recognized by these constructs is localized to region aa 255-275 of the F protein (Table 1).

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Construct	Binding to rF	Competition Binding to rF				
	EC ₅₀ (M)	RSMU19 mAb (aa 429)*	B4 mAb (aa 268, 272, 275)			
Fab19	10-9	_	+			
mAb 19A	10-9	not tested	not tested			

+

Table 1: Viral F Protein Epitope Recognized by mAb 19B

mAb 19B

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5 The mAb 19B also showed specific binding to RSV infected cells indicating recognition of the F protein as displayed in its native form. VERO cells infected with approximately 50 TCID50 RS Long virus were fixed in 90% methanol when CPE reached > 90% and were used as antigen in the ELISA format described above. Binding of biotinylated mAb 19B was detected with HRP-labelled -Streptavidin. In 10 this assay, the EC50 for mAb 19B was 34 +/- ng/ml.

Example H In vitro antiviral activity of the Hu19 Antibodies

The ability of Fab fragments to inhibit virus-induced cell fusion was determined using a modification of the in vitro microneutralization assay described by Beeler et al (J. of Virology 63: 2941-2950 (1989)). In this assay, 50 ul of RS Long strain virus (approximately 100 TCID₅₀/well; American Type Culture Collection ATCC VR-26) were mixed with 0.1 ml VERO cells (5 x 10^3 /well; ATCC 20 CCL-81) in Minimum Essential Media (MEM) containing 2% FCS, for 4 hours at 37°C, 5% CO₂. Serial two-fold dilution (in duplicate) of test samples (50 ul) were then added to wells containing virus-infected cells. Control cultures contained cells incubated with virus only (positive virus control) or cells incubated with media alone. Cultures were incubated at 37°C in 5% CO2 for 6 days at which time 25 cytopathic effects (CPE) in virus control wells were $\geq 90\%$. Neutralization assays

^{*} amino acid residues critical for antigen recognition

were performed as described above except that serial dilutions of test samples were mixed with 100 TCID_{50} of RS virus (50 ul each) for 2 hours at 37°C in $5\% \text{ CO}_{2}$ before the addition of VERO cells (5 x 10^{3}).

Microscopic examination for cytopathic effects were confrimed by ELISA. Media was aspirated from cultures and replaced with 50 ul of 90% methanol/0.6% H₂O₂. After 10 minutes, fixative was aspirated and plates were air dried overnight. Viral antigen was detected in the fixed cultures using biotinylated human/bovine chimeric derivative of mAb B4 (RSCHB4; 1 ug/ml), followed by HRP-labelled streptavidin (Boehringer-Mannheim) diluted 1:10,000 (each lot was titrated to determine the optimal concentration). The reaction was developed using TMBlue and stopped by addition of 1 N H₂SO₄. Absorbance was measured at 450nm (O.D.₄₅₀).

Fusion-inhibition or neutralization titers were defined as the reciprocal dilution of test sample, or concentration of antibody, which caused a 50% reduction in ELISA signal (ED₅₀) as compared to virus controls. Based on the curve generated in the ELISA by the standard virus titration, a 50% reduction in O.D.₄₅₀ in wells corresponded to \geq 90% reduction in virus titer. To determine the ED₅₀, mean absorbance for replicate cultures (per dilution of test sample) was plotted against dilution of sample. Calculation of the 50% point, defined as (mean absorbance virus-infected cells + mean absorbance uninfected cells)/2, was based on regression analysis of the dose titration.

SB 209763 is a humanized derivative of RSMU19 as described in P. R. Tempest et al., Biotechnology 9, 266-271 (1991). To determine the effects of coadministration of mAb19B and SB 209763 on *in vitro* fusion-inhibition, the antibodies were titrated alone and in combination. Antibody interactions were analyzed using MacSynergy TM II software.

The *in vitro* antiviral titers of the mAb constructs generated either by direct cloning (version A) or after introduction of various sequence modifications (versions B-D) demonstrated potent neutralization and fusion-inhibition activity against a prototype RSV Long strain (Table 2). mAb 19B was also shown to neutralize clinical isolates representing multiple antigenic variants of RSV collected over the

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1993/1994 season in the Philadelphia PA area (Table 3). When mAb 19B was coadministered with a second antibody directed to a different F protein epitope (SB 209763, critical residue aa 429), the effect on inhibition of virus growth in infected cell cultures was additive (data not shown).

The antiviral titers of the mAb constructs were approximately 5 to 10-fold lower than the titers obtained with the corresponding Fab constructs - Fab19, Fab19A or B (Table 2). Fab19 is the original Fab protein produced directly in *E. coli* from the clone 19 plasmid, whereas Fab19A and Fab19B were derived by papain cleavage from the coresponding full length mAbs. Removal of the N-linked glycoslation site encoded within the CDR2 loop of the heavy chain by cloning had no effect on the overall antiviral activity of the mAb (Table 2; construct C compared to A and B). In addition, enrichment of the mAb19B construct for normally glycosylated antibody did not significantly alter the *in vitro* fusion-inhibition titer (Table 4). However, enrichment for the glycovariant Fab fragment resulted in a 2 to 10-fold reduction in *in vitro* antiviral activity compared to normally glycosylated Fab fragment (Table 4).

Table 2: Antiviral Activity of 19A, 19B, 19C, and 19D Constructs Against RS Long strain virus

Construct	Neutralization EC ₅₀ , ug/ml	Fusion-Inhibition EC ₅₀		
		(ug/ml)	(nM)	
Fab19	$0.34 \pm 0.25*$	0.22	4.4	
Fab19A	not tested	0.16	33	
Fab19B	not tested	0.12 ± 0.06	2.4	
mAb 19A	2.2	2.8 ± 1.9	18.9	
mAb 19B	1.5	2.3 ± 1.9	15.3	
mAb 19C	not tested	2.4	16	

not tested

mAb 19D

2.6

17.3

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^{*} mean <u>+</u> standard deviation

Table 3: Fusion-Inhibition Activity of mAb 19B against Clinical Isolates of RSV

<u>Virus Isolate</u>	Fusion-Inhibition Titer EC ₅₀ (ug/ml)					
	mAb19B	SB 209763				
RS Long (prototype A1)	2.3 ± 1.9	1.3 ± 0.8				
RS 9320 (prototype B1)	0.59	2.5 + 1.1				
A1 - V1763	2.79	1.95				
A2 - 847	0.89	0.27				
A2 - 626	0.35	0.36				
A3 - 7062	2.65	1.67				
A4 - 6652	2.1	1.52				
B1 - 6973	1.77	2.22				
B2 - 6556	1.49	2.05				
B3 - 447	1.78	1.7				

Table 4: Antiviral Activity of 19B Glycovariants

Construct	c _e glycovariant*	Fusion-Inhibition Titer EC50 (ug/ml)
mAb 19B	40%	2.5 <u>+</u> 1.5
Fraction A	< 5%	1.8 ± 0.8
Fraction B	40%	3.8 <u>+</u> 0.9
Fab19B	< 10%	0.12 <u>+</u> 0.06
Fraction A	1%	0.89
Fraction B	94%	1.5 <u>+</u> 0.2
Fraction C	99%	3.7

^{*} mAb or Fab fragments were untreated or run on MonoQ (Mab) or MonoS (Fab) columns to separate glycosylated versus minimally glycosylated forms in the varible region.

Example I

In vivo Activity of mAb 19B; Prophvlaxis and Therapy in Balb/c Mouse Model.

Balb/c mice (5/group) were inoculated intraperitoneally with doses ranging from 0.06 mg/kg to 5 mg/kg of mAb 19B either 24 hours prior (prophylaxis) or 4 days after (therapy) intranasal infection with 10^5 PFU of the A2 strain of human RSV. Mice were sacrificed 5 days after infection. Sera was obtained to determine antibody levels and lungs were homogenized to determine virus titers. Virus was undetectable in the lungs of mice treated prophylactically with ≥ 1.25 ug mAb 19B, and corresponding serum concentrations of ≥ 5 ug/ml (Table 5). Higher doses of mAb 19B were required for complete viral clearance when mAb was administered therapeutically (5 mg/kg).

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Table 5: mAb 19B Prophylaxis and Therapy in Balb/c Mice

		Proph	ylaxis	Therapy			
Treatment Dose (mg/kg		Lung Virus Titer (log10/g lung)	Serum Concentration (ug/ml)	Lung Virus Titer (log10/g lung)	Serum Concentration (ug/ml)		
mAb 19B	5	≤1.7	15.6	≤1.7	13.2		
	1.25	<u>≤</u> 1.7	5.0	2.5 ± 0.4	2.1		
	0.31	3.2 <u>+</u> 0.3	0.79	3.8 ± 0.2	0.61		
	0.06	3.8 <u>+</u> 0.6	0.17	4.5 ± 0.1	0.08		
PBS	-	5.2 ± 0.1	≤0.02	4.7 ± 0.3	≤0.036		

The results of examples G through I establish that the Hu19 antibodies have potent antiviral activity in vitro against a broad range of native RSV isolates of both type A and B, and show prophylactic and therapeutic efficacy in vivo in animal models. Thus, the Hu19 antibodies, most preferably Hu19C or Hu19D, are candidates for therapeutic, prophylactic, and diagnostic application in man.

PROMETERS IN THE

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	Exited, Destinations
	sw⊢et, kaymond A.
10	(.1) TITLE OF THE INVENTION: Human Mondolonal Antibodies
	(111) NUMBER OF SEQUENCES: 30
	(iv) Cürrespondence Address:
15	(A) ADDRESSEE: UmithKline Beecham Curporation
	(B) STREET: "04 Swedeland Road
	(C) CITY: King of Prussia
	(D) STATE: PA
	(E) COUNTRY: U.S.A.
2()	(F) ZIP: 19046
	(v) COMPUTER READABLE FORM:
	(A) MEDIUM TYPE: Diskette
	(B) COMPUTER: IBM Compatible
25	(C) OPERATING SYSTEM: DOC
	(D) SOFTWARE: FastSEQ for Windows Version 2.0
	(V1) CURRENT APPLICATION DATA:
	(A) APPLICATION NUMBER:
3()	(B) FILING DATE:
	(C) CLASSIFICATION:
	(vii) PRIOR APPLICATION DATA:
	(A) APPLICATION NUMBERS + % % 149
35	(B) FILING DATE: 01-Net

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(viii) ATTORNEY/AGENT INFORMATION:
              (A) NAME: Geiger, Kathleen
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              (B) REGISTRATION NUMBER: 35,880
              (C) REFERENCE/DOCKET NUMBER: P50504
            (ix) TELECOMMUNICATION INFORMATION:
              (A) TELEPHONE: 610-270-5968
10
              (B) TELEFAX: 610-270-5090
              (C) TELEX:
               (2) INFORMATION FOR SEQ ID NO:1:
15
            (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 17 amino acids
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
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              (D) TOPOLOGY: linear
            (ii) MOLECULE TYPE: protein
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
25
      Ser Ile Thr Gly Gly Ser Asn Gly Ile Asn Tyr Ala Asp Ser Val Lys
       1
                                           10
                                                                15
      Arg
30
                (2) INFORMATION FOR SEQ ID NO:2:
             (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 17 amino acids
35
               (B) TYPE: amino acid
               (C) STRANDEDNESS: single
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     Wer lie Im. Bly Bly Ber Abh Bly lie Ash Tyr Ber Asposer Val Byr
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             (..) INFORMATION FOR SEQ ID NO:3:
          (i) SEQUENCE CHARACTERISTICS:
15
           (A) LENGTH: 17 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear
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         (ii) MOLECULE TYPE: protein
           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
     Ser Ile Thr Gly Gly Ser Ash Gly Ile Gln Tyr Ser Asp Ser Val Lys
25
                               10
                                                      1.5
     Arg
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           (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 98 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
35
            (D) TOPOLOGY: linear
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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

5 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly 5 1 10 15 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr 20 25 30 Glu Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val 10 35 40 45 Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val 55 60 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr 70 75 15 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys 85 90 95 Ala Arg

- 20 (2) INFORMATION FOR SEQ ID NO:5:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 139 amino acids
 - (B) TYPE: amino acid
- (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: protein
- 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly

1 5 10 15

Val His Ser Glu Val Gln Leu Leu Glu Val Glu Ser Gly Gly Leu

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	, ~	Asp	Terr	Val	Lyl:	Siy	Arņ	Phe	Trii	T 1 +0	Ser	Àl I	Acy	Arm	Ala	Lyr
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	Aan	3-1	Leu	Tyr	Leu	G.n	Met	Asn	Se:	Letu	m.	A14	314	AJP	Thr	Ala
10				104					105					1.1		
	Val	Tyr	Туг	CyC	Ala	Thr	Ala	$\Gamma(\Sigma,\gamma)$	Ile	Ala	[) <u>Y</u> =	Pr ·	Tyr	Phe	Asp	Hi.
			115					120					1 1/2 5			
	Trp	Gly	Gin	Gly	Thr	Leu	Val	Thr	Val	. See I	Ser					
		130					135									
15																
			(2) INI	FORM	icir.	V FOI	P JEK	1 12	N⊖ : :	<i>5</i> :					
		(i) Si	EQUEI	VCE (CHARA	ACTE	RIST	ICS:							
			(A)	LENG	GTH:	138	amıı	no a	cids							
20			(B)	TYP!	E: ar	mino	acı	7								
			(C)	STF	ANDEI	DNES:	S: 3	ınal.	÷							
			(D)	TOP	olog'	Y: 1.	inea	·								
		(ii) !	MOLE	CULE	TYP	E: p	rote.	in							
25																
		(:	xi) .	SEQU!	ENCE	DES	CRIP'	TION	: SE	Q ID	NO:	ő :				
	Met	Ğlu	Phe	Gly	Leu	Ser	Trp	Val	Phe	Leu	Val	Ala	Deu	Leu	Arg	Gly
	1				5					10					15	
30	Val	Gln	Суѕ	Gln	Val	Gln	Leu	Val	Val	Glu	Ser	Gly	Gly	Gly	Leu	Arg
				20					25					30		
	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Суз	Ala	Ala	Ser	Gly	Thr	Thr	Leu
			35					4 i)					45			
	Ser	Gly	Тут	Thr	Met	His	Trp	Wal	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu
35		E 0					5.5					50				

Glu Trp Val Ser Ser Ile Thr Gly Gly Ser Asn Phe Ile Asn Tyr Ser Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Thr Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Thr Ala Pro Ile Ala Pro Pro Tyr Phe Asp His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

- (2) INFORMATION FOR SEQ ID NO:7:
- (i) SEQUENCE CHARACTERISTICS:
- 15 (A) LENGTH: 138 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
- Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly Val Gln Cys Gln Val Gln Leu Val Val Glu Ser Gly Gly Leu Arg Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Thr Thr Leu Ser Gly Tyr Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ser Ile Thr Gly Gly Ser Asn Phe Ile Asn Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn

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           (1) INFORMATION FUR SECTION NO: 5:
10
    '13 SEQUENCE CHARACTERISTICS:
          (A: LENGTH: 138 amin/ acids
          (B) TYPE: amino acid
          (d) STRANDEDNESS: Single
          (D) TOPOLOGY: linear
15
        wii) MOLECULE TYPE: protein
         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
20
    Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
                     10
           5
    Val Glm Cys Glm Val Glm Leu Val Val Glu Ser Gly Gly Gly Leu Arg
                      25
             20
    Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Thr Thr Leu
25
    35 40 45
    Ser Gly Tyr Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
     50 55 60
    Glu Trp Val Ser Ser Ile Thr Gly Gly Ser Asn Phe Ile Gln Tyr Ser
                        75
    65 70
30
    Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asr.
                                90
                85
    Ser Leu Tyr Leu Gln Met Asn Ser Leu Thr Ala Glu Asp Thr Ala Val
                       135
             100
    Tyr Cys Ala Thr Ala Pro Ile Ala Pro Dro Tyr Phe Asp His Trp
35
     125
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Gly Gln Gly Thr Leu Val Thr Val Ser Ser 130 135 (2) INFORMATION FOR SEQ ID NO:9: 5 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 88 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single 10 (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9: 15 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly 1 10 15 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr 20 25 30 20 Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile 40 45 35 Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly 50 60 55 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro 25 75 80 70 Glu Asp Phe Ala Thr Tyr Tyr Cys 85 (2) INFORMATION FOR SEQ ID NO:10: 30 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 124 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

The Month of the Control of the Cont S. PROPER PROBLEM NO P. L. D. St. . More thy Try Service the the less the Len Val Ala Phr Ala Phr thy $oldsymbol{1}_{i}$. The state of the stat Val His Ser Gin Lei Ihr Glin Ser Er chor Ser Lew Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Thr Gin Ser Val Ser Ash 35 40 45 A 1() Phe Leu Ash Trp Tyr Iln Gin Lya Fre Hly Glu Ala Pro The Leu Leu 55 55 6C Ile Tyr App Ala Ser Thr Ser Glm Ser Gly Val Pro Ser Ard Phe Ser e5 75 75 80 Gly Ser Gly Ser Gly Met Asp Pho Ser Leu Thr Ile Jer Ser Leu Glr. 15 95 90 95 Pro Glu Asp Leu Ala Met Tyr Tyr Cys Gln Ala Ser Ile Asn Thr Pro 100 Let Phe Gly Gly Gly Thr Arg Ile Asp Met Arg Arg 20 119 120 (2) INFORMATION FOR SEQ ID NO:11: (1) SEQUENCE CHARACTERISTICS: 25 (A) LENGTH: 101 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 30 (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SE, ID NO:11: Met Arq Val Pro Ala Gln Leu Leu Sly Leu Leu Leu Irp Leu Arg

	Gly	Ala	Arg	Cys	Asp	Ile	Gln	Met	Asn	Phe	Leu	Asn	Trp	Tyr	Gln	Gln
				20					25					30		
	Lys	Pro	Gly	Glu	Ala	Pro	Thr	Leu	Leu	Ile	Tyr	Asp	Ala	Ser	Thr	Ser
			35					40					45			
5	Gln	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Met	Asp
		50					55					60				
	Phe	Ser	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu	Asp	Leu	Ala	Met	Tyr
	65					70					75					80
	Tyr	Cys	Gln	Ala	Ser	Ile	Asn	Thr	Pro	Leu	Phe	Gly	Gly	Gly	Thr	Arg
10					85					90					95	
	Ile	Asp	Met	Arg	Arg											
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			(2)) INI	FORM	1OITA	V FOI	R SE(Q ID	NO : 1	12:					
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30	Pro	Δra	Glu		Lve	Val	Gln	Trn		Val	Asn	Aen	Δla	Leu	Gln	Ser
50	110	Arg	35	AIG	БуЗ	V ()	G111	40	цуз	var	p	11011	45	Dea	0111	DCI
	Glv	Aen		Gln	Glu	Ser	Val		Glu	Gln	Asp	Ser		Asp	Ser	Thr
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	ጥ ላታም		I.eu	Ser	Ser	ም ክ r		ጥ ከ r	Leu	Ser	Ive		Asp	Tyr	Glu	Lvs
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           *L INFORMATION BOX SEV IN INCLUSE
         " SEQUENCE CHARACTERISTICS:
          (A) LENGTH: lub amino acids
10
          (B) TYPE: amin. arid
          (C) STRANDEDNESS: single
          (D) Topology: linear
         (ii) MOLECULE TYPE: protein
15
        (x1) SEQUENCE DESCRIPTION: SEQ ID NO:13:
    Thr Val Ala Ala Pro Ser Val Phé Île Phé Pro Pro Ser Asp Glu Glo
          5
20
    Leu Lys Ser Gly Thr Ala Ser Val Val Cys heu Leu Asn Asn Phe Tyr
                   25
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                                       3 C
    Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
     35 4ú 45
    Gly Asn Ser Glu Ger Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
    55 50
25
    Tyr Ser Leu Ser Ser Thr Leu Thi Leu Ser Lys Ala Asp Tyr Glu Lys
    70 75
     His Lys Val Tyr Ala Cys Glu Val Inn His Gln Gly Leu Ser Leu Pro
            85
30
    Val Thr Lys Ser Phe Ash Arg Gly Gl. Tys
             100
           (D) INFORMATION FOR JE. II NJ:14:
35
        (i) SEQUENCE CHARACTERICTIAN
          (A) LENGTH: 6284 base point
```

(B) TYPE: nucleic acid(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

	GACGTCGCGG	CCGCTCTAGG	CCTCCAAAAA	AGCCTCCTCA	CTACTTCTGG	AATAGCTCAG	60
	AGGCCGAGGC	GGCCTCGGCC	TCTGCATAAA	TAAAAAAAAT	TAGTCAGCCA	TGCATGGGGC	120
10	GGAGAATGGG	CGGAACTGGG	CGGAGTTAGG	GGCGGGATGG	GCGGAGTTAG	GGGCGGGACT	180
	ATGGTTGCTG	ACTAATTGAG	ATGCATGCTT	TGCATACTTC	TGCCTGCTGG	GGAGCCTGGG	240
	GACTTTCCAC	ACCTGGTTGC	TGACTAATTG	AGATGCATGC	TTTGCATACT	TCTGCCTGCT	300
	GGGGAGCCTG	GGGACTTTCC	ACACCCTAAC	TGACACACAT	TCCACAGAAT	TAATTCCCGG	360
	GGATCGATCC	GTCGACGTAC	GACTAGTTAT	TAATAGTAAT	CAATTACGGG	GTCATTAGTT	420
15	CATAGCCCAT	ATATGGAGTT	CCGCGTTACA	TAACTTACGG	TAAATGGCCC	GCCTGGCTGA	480
	CCGCCCAACG	ACCCCCGCCC	ATTGACGTCA	ATAATGACGT	ATGTTCCCAT	AGTAACGCCA	540
	ATAGGGACTT	TCCATTGACG	TCAATGGGTG	GACTATTTAC	GGTAAACTGC	CCACTTGGCA	600
	GTACATCAAG	TGTATCATAT	GCCAAGTACG	CCCCCTATTG	ACGTCAATGA	CGGTAAATGG	660
	CCCGCCTGGC	ATTATGCCCA	GTACATGACC	TTATGGGACT	TTCCTACTTG	GCAGTACATC	720
20	TACGTATTAG	TCATCGCTAT	TACCATGGTG	ATGCGGTTTT	GGCAGTACAT	CAATGGGCGT	780
	GGATAGCGGT	TTGACTCACG	GGGATTTCCA	AGTCTCCACC	CCATTGACGT	CAATGGGAGT	840
	TTGTTTTGGC	ACCAAAATCA	ACGGGACTTT	CCAAAATGTC	GTAACAACTC	CGCCCCATTG	900
	ACGCAAATGG	GCGGTAGGCG	TGTACGGTGG	GAGGTCTATA	TAAGCAGAGC	TGGGTACGTG	960
	AACCGTCAGA	TCGCCTGGAG	ACGCCATCGA	ATTCTGAGCA	CACAGGACCT	CACCATGGGA	1020
25	TGGAGCTGTA	TCATCCTCTT	CTTGGTAGCA	ACAGCTACAG	GTGTCCACTC	CGAGGTCCAA	1080
	CTGCTCGAGG	AGTCTGGGGG	AGGCCTGGTC	AGGCCTGGCG	GGTCCCTAAG	ACTCTCGTGT	1140
	GCAGCCTCTG	GAACCACCCT	CAGTGGCTAT	ACCATGCACT	GGGTCCGCCA	GGCTCCAGGG	1200
	AAGGGGCTGG	AGTGGGTCTC	ATCCATTACT	GGAGGTAGCA	ACTTCATAAA	CTACTCAGAC	1260
	TCAGTGAAGG	GCCGATTCAC	CATCTCCAGA	GACAACGCCA	AGAACTCACT	TTATCTGCAA	1320
30	ATGAACAGCC	TGACAGCCGA	GGACACGGCT	GTCTATTATT	GTGCGACCGC	CCCTATAGCA	1380
	CCGCCCTACT	TTGACCACTG	GGGCCAGGGA	ACCCTGGTCA	CCGTCTCCTC	AGCCTCCACC	1440
	AAGGGCCCAT	CGGTCTTCCC	CCTGGCACCC	TCCTCCAAGA	GCACCTCTGG	GGGCACAGCG	1500
	GCCCTGGGCT	GCCTGGTCAA	GGACTACTTC	CCCGAACCGG	TGACCGTGTC	GTGGAACTCA	1560
	GGCGCCCTGA	CCAGCGGCGT	GCACACCTTC	CCGGCTGTCC	TACAGTCCTC	AGGACTCTAC	1620
35	TCCCTCAGCA	GCGTGGTGAC	TGTGCCCTCC	AGCAGCTTGG	GCACCCAGAC	CTACATCTGC	1680
	AACGTGAATC	ACAAGCCCAG	CAACACCAAG	GTGGACAAGA	AAGTTGAGCC	CAAATCTTGT	1740

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	31 2A 17 17	OMI MAYI	COTOCCA ROS	200ATC3A3A	222 20000	DAAAGMZAAL	
	GGGCAGCCC	GAGAMOTACA	GGTGTACACC	CTGCCCCAT	211366A75A	CCTGACCAA.;	
	AAQCAGGTCA	GCCTGACCTG	CCTGGTCAAA	GGCTTCTATC	CCAGCGAGAT	CGCCGTGGA-	
	TGGGAGAGCA	ATGGCAGCC	GGAGAACAAC	TACAAGACCA	CGCCTCCCGT	GCTGGACTO	
10	GACGGCTCCT	TETTCETETA	CAGCAAGCTC	ACCGTGGACA	AGAGCAGGTG	GCAGCAGGG:	÷ ·;
	AACGTCTTCT	CATGCTCCGT	GATGCATGAG	GCTCTGCACA	ACCACTACAC	GCAGAAGAG 1	1
	CTCTCCCTGT	CTCCGGGTAA	ATGATAGATA	TCTACGTATG	ATCAGCCTCG	ACTGTGCCTT	1
	CTAGTTGCCA	GCCATCTGTT	GTTTGCCCCT	CCCCCGTGCC	TTCCTTGACC	CTGGAAGGTC	
	CCACTCCCAC	#GTCCTTTCU	TAATAAAATG	AGGAAATTGC	ATCGCATTGT	CTGAGTAGGI	* * **
15	GTCATTCTAT	TTTGGGGGGGT	GGGGTGGGGC	AGGACAGCAA	GGGGGAGGAT	TGGGAAGACA	, r
	ATAGCAGGCA	TGCTGGGGAT	GCGGTGGGCT	CTATGGAACC	AGCTGGGGCT	CGACAGCGCT	, ,
	GGATCTCCCG	ATCCCCAGCT	TTGCTTCTCA	ATTTCTTATT	TGCATAATGA	GAAAAAAGG	
	TTAATTAAAA	TTAACACCAA	TTCAGTAGTT	GATTGAGCAA	ATGCGTTGCC	AAAAAGGATS	2
	CTTTAGAGAC	AGTGTTCTCT	GCACAGATAA	GGACAAACAT	TATTCAGAGG	GAGTACCCAG	
20	AGCTGAGACT	CCTAAGCCAG	TGAGTGGCAC	AGCATTCTAG	GGAGAAATAT	GCTTGTCAT	
	ACCGAAGCCT	GATTCCGTAG	AGCCACACCT	TGGTAAGGGC	CAATCTGCTC	ACACAGGATA	¥
	GAGAGGGCAG	GAGCCAGGGC	AGAGCATATA	AGGTGAGGTA	GGATCAGTTG	CTCCTCACAI	•
	TTGCTTCTGA	CATAGTTGTG	TTGGGAGCTT	GGATAGCTTG	GACAGCTCAG	GGCTGCGATT	· : <u>-</u>
	TCGCGCCAAA	CTTGACGGCA	ATCCTAGCGT	GAAGGCTGGT	AGGATTTTAT	cadegarse i	* _ =
25	ATCATGGTTC	GACCATTGAA	CTGCATCGTC	GCCGTGTCCC	AAAATATGGG	GATTGGCAA :	
	AACGGAGACC	TACCCTGGCC	TCCGCTCAGG	AACGAGTTCA	AGTACTTCCA	AAGAATGA."	
	ACAACCTCTT	CAGTGGAAGG	TAAACAGAAT	TGGTGATTA	TGGGTAGGAA	AACCTGGTT	
	TCCATTCCTG	AGAAGAATCG	ACCTTTAAAG	GACAGAATTA	ATATAGTTCT	CAGTAGAGAA	
	CTCAAAGAAC	CACCACGAGG	AGCTCATTTT	CTTGCCAAAA	GTTTGGATGA	TGCCTTAAGA	· -
30	CTTATTGAAC	AACCGGAATT	GGCAAGTAAA	STAGACATĞĞ	TTTGGATAGT	CGGAGGCAGT	1/2
	TCTGTTTACC	AGGAAGCCAT	GAATCAACDA	BOCCACCTTA	GACTCTTTGT	GACAAGGAT 1	* * * * * * * * * * * * * * * * * * * *
	ATGCAGGAAT	TTGAAAGTGA	CACGTTTTTC	JCAGAAATTG	ATTTGGGGAA	ATATAAACTT	* P* **
	CTCCCAGAAT	ACCCAGGCGT	CCTCTCTGAG	GTCCAGGAGG	AAAAAGGCAT	CAAGTATAA ?	. ~
	TTTGAAGTCT	ACGAGAAGAA	AGACTAACAG	UAAGATGCTT	TCAAGTTCTC	TGCTCCCTT '	
35	CTAAAGCTAT	GCATTTTTAT	AAGACCATGG	GACTTTTGCT	GGCTTTAGAT	CAGCCTCGAG	7 7 4
	TGTGCCTTCT	AGTTGCCAGC	CATCTGTTGT	TTGGCCCTCC	CCCGTGCCTT	CCTTGACCCT	5.7

	GGAAGGTGCC	ACTCCCACTG	TCCTTTCCTA	ATAAAATGAG	GAAATTGCAT	CGCATTGTCT	3960
	GAGTAGGTGT	CATTCTATTC	TGGGGGGTGG	GGTGGGGCAG	GACAGCAAGG	GGGAGGATTG	4020
	GGAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT	ATGGAACCAG	CTGGGGCTCG	4080
	ATCGAGTGTA	TGACTGCGGC	CGCGATCCCG	TCGAGAGCTT	GGCGTAATCA	TGGTCATAGC	4140
5	TGTTTCCTGT	GTGAAATTGT	TATCCGCTCA	CAATTCCACA	CAACATACGA	GCCGGAAGCA	4200
	TAAAGTGTAA	AGCCTGGGGT	GCCTAATGAG	TGAGCTAACT	CACATTAATT	GCGTTGCGCT	4260
	CACTGCCCGC	TTTCCAGTCG	GGAAACCTGT	CGTGCCAGCT	GCATTAATGA	ATCGGCCAAC	4320
	GCGCGGGGAG	AGGCGGTTTG	CGTATTGGGC	GCTCTTCCGC	TTCCTCGCTC	ACTGACTCGC	4380
	TGCGCTCGGT	CGTTCGGCTG	CGGCGAGCGG	TATCAGCTCA	CTCAAAGGCG	GTAATACGGT	4440
10	TATCCACAGA	ATCAGGGGAT	AACGCAGGAA	AGAACATGTG	AGCAAAAGGC	CAGCAAAAGG	4500
	CCAGGAACCG	TAAAAAGGCC	GCGTTGCTGG	CGTTTTTCCA	TAGGCTCCGC	CCCCTGACG	4560
	AGCATCACAA	AAATCGACGC	TCAAGTCAGA	GGTGGCGAAA	CCCGACAGGA	CTATAAAGAT	4620
	ACCAGGCGTT	TCCCCCTGGA	AGCTCCCTCG	TGCGCTCTCC	TGTTCCGACC	CTGCCGCTTA	4680
	CCGGATACCT	GTCCGCCTTT	CTCCCTTCGG	GAAGCGTGGC	GCTTTCTCAA	TGCTCACGCT	4740
15	GTAGGTATCT	CAGTTCGGTG	TAGGTCGTTC	GCTCCAAGCT	GGGCTGTGTG	CACGAACCCC	4800
	CCGTTCAGCC	CGACCGCTGC	GCCTTATCCG	GTAACTATCG	TCTTGAGTCC	AACCCGGTAA	4860
	GACACGACTT	ATCGCCACTG	GCAGCAGCCA	CTGGTAACAG	GATTAGCAGA	GCGAGGTATG	4920
	TAGGCGGTGC	TACAGAGTTC	TTGAAGTGGT	GGCCTAACTA	CGGCTACACT	AGAAGGACAG	4980
	TATTTGGTAT	CTGCGCTCTG	CTGAAGCCAG	TTACCTTCGG	AAAAAGAGTT	GGTAGCTCTT	5040
20	GATCCGGCAA	ACAAACCACC	GCTGGTAGCG	GTGGTTTTTT	TGTTTGCAAG	CAGCAGATTA	5100
	CGCGCAGAAA	AAAAGGATCT	CAAGAAGATC	CTTTGATCTT	TTCTACGGGG	TCTGACGCTC	5160
	AGTGGAACGA	AAACTCACGT	TAAGGGATTT	TGGTCATGAG	ATTATCAAAA	AGGATCTTCA	5220
	CCTAGATCCT	TTTAAATTAA	AAATGAAGTT	ТТАААТСААТ	CTAAAGTATA	TATGAGTAAA	5280
			TGCTTAATCA				5340
25			TGACTCCCCG				5400
	TACCATCTGG	CCCCAGTGCT	GCAATGATAC	CGCGAGACCC	ACGCTCACCG	GCTCCAGATT	5460
			GCCGGAAGGG				5520
			AATTGTTGCC				5580
20			GCCATTGCTA				5640
30			GGTTCCCAAC				5700
			TCCTTCGGTC				5760
			ATGGCAGCAC				5820
			GGTGAGTACT				5880
2.5			CCGGCGTCAA				5940
35			GGAAAACGTT				6000
	CGCTGTTGAG	ATCCAGTTCG	ATGTAACCCA	CTCGTGCACC	CAACTGATCT	TCAGCATCTT	6060

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TRANTITINA NI NA SINSTITUTO ING ITAMA NAAN AAA NAGI WAA SI IN WAAAA DISIN IN HAAAAAA AA IN NI NA WAAAAA AA AA
      SHATAA 1000 WA WA 151AAA PETT HAATA 1 10ATA TETE 1 1000TT MAA TA FATT WAA 1 4 14
      PATTIADIA PRITATEST TIATSAS CERTA MĀRIC TWAT GARI TA MAMAMIN.
      AADAAATA GU 1971 WAADAA AWADTOO DAAAAAGTOO AGOO
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               . INFORMATINF BUTE, INNOTES
            : DEQUENCE CHARACTERISTICS:
             (A) LEMSTH: of amine Acids
10
             B) TYPE: amino acid
             (T) STRANDEDNESS: single
             (D) TOPOLOGY: linear
            (11) MOLECULE TYPE: protein
15
           (x1) SEQUENCE DESCRIPTION: SET ID NO:15:
     Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
                                      1.0
20
     Var His Ser Glu Val Gln Leu Leu Glu Val
                 23
                                     25
              (2) INFORMATION FOR SEQ ID NO:16:
25
           (1) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 4 amino acids
             (B) TYPE: amino acid
              (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear
30
           (ii) MOLECULE TYPE: protein
           (x1) SEQUENCE DESCRIPTION: SEO ID NO:16:
35 Ser Pro Gly Lys
```

	(2) INFORMATION FOR SEQ ID NO:17:	
	(i) SEQUENCE CHARACTERISTICS:	
5	(A) LENGTH: 25 amino acids	
	(B) TYPE: amino acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
10	(ii) MOLECULE TYPE: protein	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:	
	Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly	
15	1 5 10 15	
	Val His Ser Glu Leu Thr Gln Ser Pro	
	20 25	
20	(2) INFORMATION FOR SEQ ID NO:18:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 5681 base pairs	
	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
25	(D) TOPOLOGY: linear	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:	
30	GACGTCGCGG CCGCTCTAGG CCTCCAAAAA AGCCTCCTCA CTACTTCTGG AATAGCTCAG	60
	AGGCCGAGGC GGCCTCGGCC TCTGCATAAA TAAAAAAAAT TAGTCAGCCA TGCATGGGGC	120
	GGAGAATGGG CGGAACTGGG CGGAGTTAGG GGCGGGATGG GCGGAGTTAG GGGCGGGACT	180
	ATGGTTGCTG ACTAATTGAG ATGCATGCTT TGCATACTTC TGCCTGCTGG GGAGCCTGGG	240
	GACTTTCCAC ACCTGGTTGC TGACTAATTG AGATGCATGC TTTGCATACT TCTGCCTGCT	300

GGGGAGCCTG GGGACTTTCC ACACCCTAAC TGACACACAT TCCACAGAAT TAATTCCCGG

GGATCGATCC GTCGACGTAC GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT

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	PAK TALKI.	THTAT MATAT	150 754 377 1	and all man	5 (3/3m 13 5 7 3 5 7 25. 27. 2025 1 225	MANUALAN I I	
5		ATTATON IA	TACATEACT	TOTAL BONDS	TURNITATION	GAMITACAT	•
	737 7777773	POATOGUTAT	TACCATORTS	AT STATISTICS	GOCASTACAT	AATSAG JAD	-
	COATABIONT	TTGACTCACG	GGGATTTCCA	ACTOPPOACO	CCATTGACCI	DAATTIGAGT	-, <u>-</u>
	TTGTTTTTGGC	ACCAAAATCA	ACGGGACTTT	CCAAAATGTC	GTAACAACT;	CGCCCCATTA	S4 - 25 - 1
	ACGUAAATGG	GCGGTAGGCG	TGTACGGTGG	GAGGTCTATA	TAAGCAGAG!	TGGGTACGT:	15
10	AACCGTCAGA	TCGCCTGGAG	ACGCCATCGA	ATTCTGAGCA	CACAGGACCT	CACCATGGGA	101
	TGGAGCTGTA	TCATCCTCTT	CTTGGTAGCA	ACAGCTACAG	GTGTCCACT	CGAGCTCAC 1	in A
	CAGTOTOCAT	CCTCCCTGTC	TGCATCTGTA	GGAGACAGAG	TCACCATCAC	TTGCPAGGCA	114
	ACTCAGAGTG	TTAGTAACTT	TTTAAATTGG	TATCAGCAGA	AGCCAGGGĞA	AGCCCCTACR	220
	CTCCTGATCT	ATGATGCATC	CACTTCGCAA	AGTGGGGTCC	CATCAAGGTT	CAGTGGCAGI	16
15	GGATCTGGGA	TGGATTTCAG	TCTCACCATC	AGCAGTCTGC	AGCCTGAAJA	TCTTGCAATA	
	TATTACTGTC	AAGCGAGTAT	CAATACCCCG	CTTTTCGGCG	GAGGGACCAG	AATAGATATG	1481
	AGACGAACTG	TGGCTGCACC	ATCTGTCTTC	ATCTTCCCGC	CATCTGATGA	GCAGTTGAAA	144 =
	TCTGGAACTG	CCTCTGTTGT	GTGCCTGCTG	AATAACTTCT	ATCCCAGAGA	GGCCAAAGTA	195
	CAGTGGAAGG	TGGATAACGC	CCTCCAATCG	GGTAACTCCC	AGGAGAGTGT	CACAGAGCAG	. -
20	GACAGCAAGG	ACAGCACCTA	CAGCCTCAGC	AGCACCCTGA	CGCTGAGCAA	AGCAGACTAL	. b.i.
	GAGAAACACA	AAGTCTACGC	CTGCGAAGTC	ACCCATCAGG	GCCTGAGCTT	GCCCGTCACA	. 5 = -
	AAGAGCTTCA	ACAGGGGAGA	GTGTTAGTGA	GATGATCCTC	TAGAGTCATC	TACGTATGAT	1 4 1
	CAGCCTCGAC	TGTGCCTTCT	AGTTGCCAGC	CATCTGTTGT	TTGCCCCTCC	CCCGTGCCTT	12.
	CCTTGACCCT	GGAAGGTGCC	ACTCCCACTG	TCCTTTCCTA	ATAAAATGAG	GAAATTGCAT	$1 \sim \varepsilon_{ *}$
25	CGCATTGTCT	GAGTAGGTGT	CATTCTATTC	TGGGGGGTGG	GGTGGGGCAG	GACAGCAAGG	192
	GGGAGGATTG	GGAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT	ATGGAACCA-3	
	CTGGGGCTCG	ACAGCTCGAG	CTAGCTTTGC	TTCTCAATTT	CTTATTTGCA	TAATGAGAAA	_047
	AAAAGGAAAA	TTAATTTTAA	CACCAATTCA	STAGTTGATT	GAGCAAATGC	GTTGCCAAAA	10
	AGGATGCTTT	AGAGACAGTG	TTCTCTGCAC	AGATAAGGAC	AAACATTATT	CAGAGGGAGT	1 5
30	ACCCAGAGCT	GAGACTCCTA	AGCCAGTGAG	TGGCACAGCA	TTCTAGGGAG	AAATATGCTT	2220
	GTCATCACCG	AAGCCTGATT	CCGTAGAGCC	ACACCTTGGT	AAGGGCCAAT	CTGCTCACAU	<u> </u>
	AGGAT'AGAGA	GGGCAGGAGC	CAGGGCAGAG	CATATAAGGT	GAGGTAGGAT	CAGTIGCTCI	1343
	TCACATTTGC	TTCTGACATA	GTTGTGTTGG	GAGCTTGGAT	CGATCCACCA	TGGTTGAACA	2400
	AGATGGATTG	CACGCAGGTT	CTCCGGCCGC	TTGGGTGGAG	AGGCTATTCG	GCTATGACTU	2460
35	GGCACAACAG	ACAATCGGCT	GCTCTGATGC	CGCCGTGTTC	CGGCTGTCAG	CGCAGGGGCG	2520
	CCCGGTTCTT	TTTGTCAAGA	CCGACCTGTC	CEGTGCCCTG	AATGAACTGC	AGGACGAGGC	ិ្ទទួន្ ^ន

	AGCGCGGCTA	TCGTGGCTGG	CCACGACGGG	CGTTCCTTGC	GCAGCTGTGC	TCGACGTTGT	2640
	CACTGAAGCG	GGAAGGGACT	GGCTGCTATT	GGGCGAAGTG	CCGGGGCAGG	ATCTCCTGTC	2700
	ATCTCACCTT	GCTCCTGCCG	AGAAAGTATC	CATCATGGCT	GATGCAATGC	GGCGGCTGCA	2760
	TACGCTTGAT	CCGGCTACCT	GCCCATTCGA	CCACCAAGCG	AAACATCGCA	TCGAGCGAGC	2820
5	ACGTACTCGG	ATGGAAGCCG	GTCTTGTCGA	TCAGGATGAT	CTGGACGAAG	AGCATCAGGG	2880
	GCTCGCGCCA	GCCGAACTGT	TCGCCAGGCT	CAAGGCGCGC	ATGCCCGACG	GCGAGGATCT	2940
	CGTCGTGACC	CATGGCGATG	CCTGCTTGCC	GAATATCATG	GTGGAAAATG	GCCGCTTTTC	3000
	TGGATTCATC	GACTGTGGCC	GGCTGGGTGT	GGCGGACCGC	TATCAGGACA	TAGCGTTGGC	3060
	TACCCGTGAT	ATTGCTGAAG	AGCTTGGCGG	CGAATGGGCT	GACCGCTTCC	TCGTGCTTTA	3120
10	CGGTATCGCC	GCTCCCGATT	CGCAGCGCAT	CGCCTTCTAT	CGCCTTCTTG	ACGAGTTCTT	3180
	CTGAGCGGGA	CTCTGGGGTT	CGAAATGACC	GACCAAGCGA	CGCCCAACCT	GCCATCACGA	3240
	GATTTCGATT	CCACCGCCGC	CTTCTATGAA	AGGTTGGGCT	TCGGAATCGT	TTTCCGGGAC	3300
	GCCGGCTGGA	TGATCCTCCA	GCGCGGGGAT	CTCATGCTGG	AGTTCTTCGC	CCACCCCAAC	3360
	TTGTTTATTG	CAGCTTATAA	TGGTTACAAA	TAAAGCAATA	GCATCACAAA	TTTCACAAAT	3420
15	AAAGCATTTT	TTTCACTGCA	TTCTAGTTGT	GGTTTGTCCA	AACTCATCAA	TGTATCTTAT	3480
	CATGTCTGGA	TCGCGGCCGC	GATCCCGTCG	AGAGCTTGGC	GTAATCATGG	TCATAGCTGT	3540
	TTCCTGTGTG	AAATTGTTAT	CCGCTCACAA	TTCCACACAA	CATACGAGCC	GGAAGCATAA	3600
	AGTGTAAAGC	CTGGGGTGCC	TAATGAGTGA	GCTAACTCAC	ATTAATTGCG	TTGCGCTCAC	3660
	TGCCCGCTTT	CCAGTCGGGA	AACCTGTCGT	GCCAGCTGCA	TTAATGAATC	GGCCAACGCG	3720
20	CGGGGAGAGG	CGGTTTGCGT	ATTGGGCGCT	CTTCCGCTTC	CTCGCTCACT	GACTCGCTGC	3780
	GCTCGGTCGT	TCGGCTGCGG	CGAGCGGTAT	CAGCTCACTC	AAAGGCGGTA	ATACGGTTAT	3840
	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	ACATGTGAGC	AAAAGGCCAG	CAAAAGGCCA	3900
	GGAACCGTAA	AAAGGCCGCG	TTGCTGGCGT	TTTTCCATAG	GCTCCGCCCC	CCTGACGAGC	3960
	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT	GGCGAAACCC	GACAGGACTA	TAAAGATACC	4020
25	AGGCGTTTCC	CCCTGGAAGC	TCCCTCGTGC	GCTCTCCTGT	TCCGACCCTG	CCGCTTACCG	4080
	GATACCTGTC	CGCCTTTCTC	CCTTCGGGAA	GCGTGGCGCT	TTCTCAATGC	TCACGCTGTA	4140
	GGTATCTCAG	TTCGGTGTAG	GTCGTTCGCT	CCAAGCTGGG	CTGTGTGCAC	GAACCCCCCG	4200
	TTCAGCCCGA	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT	TGAGTCCAAC	CCGGTAAGAC	4260
	ACGACTTATC	GCCACTGGCA	GCAGCCACTG	GTAACAGGAT	TAGCAGAGCG	AGGTATGTAG	4320
30	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC	CTAACTACGG	CTACACTAGA	AGGACAGTAT	4380
	TTGGTATCTG	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	AAGAGTTGGT	AGCTCTTGAT	4440
	CCGGCAAACA	AACCACCGCT	GGTAGCGGTG	GTTTTTTGT	TTGCAAGCAG	CAGATTACGC	4500
	GCAGAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	TACGGGGTCT	GACGCTCAGT	4560
	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG	TCATGAGATT	ATCAAAAAGG	ATCTTCACCT	4620
35	AGATCCTTTT	AAATTAAAA	TGAAGTTTTA	AATCAATCTA	AAGTATATAT	GAGTAAACTT	4680
	GGTCTGACAG	TTACCAATGC	TTAATCAGTG	AGGCACCTAT	CTCAGCGATC	TGTCTATTTC	4740

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      TGTTATCAUT CATGGTTATG GCAGCACTGU ATAATTCTCT TACTGTCATO PRATCCGTAA
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      GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG TETATGCGGT
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     SACCGAGTTG CTCTTGCCCG GCGTCAATAC GGGATAATAC CGCGCCACAT AGCAGAACTT
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      TAAAAGTGCT CATCATTGGA AAACGTTCTT CGGGGGGAAA ACTCTCAAGG AFCTTACCGC
      TGTTGAGATC CAGTTCGATG TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA
                                                                            546.
      CTTTCACCAG CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA AAAAAGGGAA
                                                                            5524
      TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT TATTGAAGCA
                                                                           5584
15
     TTTATCAGGG TTATTGTCTG ATGAGCGGAT ACATATTTGA ATGTATTTAG AAAAATAAAC
                                                                            5040
      AAATAGGGGT TOOGCGCACA TTTCCCCGAA AAGTGCCACC T
                                                                            5691
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(2) INFORMATION FOR SEQ ID NO:19:

20 (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

30 Leu Pro Val Thr Lys Ser Phe Akh Arg Gly Glu Cys

1 5 10

(2) INFORMATION FOR SEQ ID NO:20:

35 (i) sequence characteristics:

(A) LENGTH: 1427 base pairs

(B) TYPE: nucleic acid(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

	GAATTCGGTA	CCATGGAGTT	TGGGCTGAGC	TGGGTTTTCC	TCGTGGCTCT	TTTAAGAGGT	60
10	GTCCAGTGTC	AGGTGCAGCT	GGTGGAGTCT	GGGGGAGGCC	TGGTCAGGCC	TGGCGGGTCC	120
	CTAAGACTCT	CGTGTGCAGC	CTCTGGAACC	ACCCTCAGTG	GCTATACCAT	GCACTGGGTC	180
	CGCCAGGCTC	CAGGGAAGGG	GCTGGAGTGG	GTCTCATCCA	TTACTGGAGG	TAGCAACTTC	240
	ATAAACTACT	CAGACTCAGT	GAAGGGCCGA	TTCACCATCT	CCAGAGACAA	CGCCAAGAAC	300
	TCACTTTATC	TGCAAATGAA	CAGCCTGACA	GCCGAGGACA	CGGCTGTCTA	TTATTGTGCG	360
	ACCGCCCCTA	TAGCACCGCC	CTACTTTGAC	CACTGGGGCC	AGGGAACCCT	GGTCACCGTC	420
15	TCCTCAGCCT	CCACCAAGGG	CCCATCGGTC	TTCCCCCTGG	CACCCTCCTC	CAAGAGCACC	480
	TCTGGGGGCA	CAGCGGCCCT	GGGCTGCCTG	GTCAAGGACT	ACTTCCCCGA	ACCGGTGACC	540
	GTGTCGTGGA	ACTCAGGCGC	CCTGACCAGC	GGCGTGCACA	CCTTCCCGGC	TGTCCTACAG	600
	TCCTCAGGAC	TCTACTCCCT	CAGCAGCGTG	GTGACCGTGC	CCTCCAGCAG	CTTGGGCACC	660
	CAGACCTACA	TCTGCAACGT	GAATCACAAG	CCCAGCAACA	CCAAGGTGGA	CAAGAAAGTT	720
20	GAGCCCAAAT	CTTGTGACAA	AACTCACACA	TGCCCACCGT	GCCCAGCACC	TGAACTCCTG	780
	GGGGGACCGT	CAGTCTTCCT	CTTCCCCCCA	AAACCCAAGG	ACACCCTCAT	GATCTCCCGG	840
	ACCCCTGAGG	TCACATGCGT	GGTGGTGGAC	GTGAGCCACG	AAGACCCTGA	GGTCAAGTTC	900
	AACTGGTACG	TGGACGGCGT	GGAGGTGCAT	AATGCCAAGA	CAAAGCCGCG	GGAGGAGCAG	960
	TACAACAGCA	CGTACCGGGT	GGTCAGCGTC	CTCACCGTCC	TGCACCAGGA	CTGGCTGAAT	1020
25	GGCAAGGAGT	ACAAGTGCAA	GGTCTCCAAC	AAAGCCCTCC	CAGCCCCCAT	CGAGAAAACC	1080
	ATCTCCAAAG	CCAAAGGGCA	GCCCCGAGAA	CCACAGGTGT	ACACCCTGCC	CCCATCCCGG	1140
	GATGAGCTGA	CCAAGAACCA	GGTCAGCCTG	ACCTGCCTGG	TCAAAGGCTT	CTATCCCAGC	1200
	GACATCGCCG	TGGAGTGGGA	GAGCAATGGG	CAGCCGGAGA	ACAACTACAA	GACCACGCCT	1260
30	CCCGTGCTGG	ACTCCGACGG	CTCCTTCTTC	CTCTACAGCA	AGCTCACCGT	GGACAAGAGC	1320
	AGGTGGCAGC	AGGGGAACGT	CTTCTCATGC	TCCGTGATGC	ATGAGGCTCT	GCACAACCAC	1380
	TACACGCAGA	AGAGCCTCTC	CCTGTCTCCG	GGTAAATGAT	AGATATC		1427

(2) INFORMATION FOR SEQ ID NO:21:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 amino acids

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               THE OTEANNEL NEW TO A LINE TO
              I a I to L Tre Library
         The Mark Tolk Type protein
           WELL OF TENDE DESCRIPTION: SEQ IN NOTELLY
      Mer Hu Phe Jly Leu .er Irp Val Phe Leu Val Ala Leu Leu Arg Gly
1()
                                        1 %
      Val Gln Cys Gln Val Gln Leu Val
               (2) INFORMATION FOR SEQ ID NO.LL:
15
            ·i) sequence characteristics:
             (A) LENGTH: /32 pase pairs
             (B) TYPE: nucleic acid
             (C) STRANDEDNESS: single
20
             (D) TOPOLOGY: linear
            (x1) SEQUENCE DESCRIPTION: SEQ ID NO:22:
25
     GAATTOCATG GACATGAGGG TOCCOGCTCA GITCCTAGGG CTCCTGCTGC TCTGGCTCIG
      AGGTGCCAGA TGTGACATCC AGATGACCCA STCTCCATCC TCCCTGTCTG CATCTGTAGS
      AGACAGAGTO ACCATCACTT GCCGGGCAAS TUAGAGTGTT AGTAACTTTT TAAATTGGTA
                                                                           i Ave
      TCAGCAGAAG CCAGGGGAAG CCCCTACGCT COTGATCTAT GATGCATCCA CTTCGCAAAR
                                                                           TGGGGTCCCA TCAAGGTTCA GTGGCAUTOD ATCTGGGATG GATTTCAGTC TCACCATCAG
                                                                          3 、 5
                                                                          300
30
      CAGTOTGCAG COTGAAGATO TTGCAATGTA TTARTGTCAA GOGAGTATCA ATACCOCGGT
                                                                          120
      TTTCGGCGGA GGGACCAGAA TAGATATGAG ANGAACTGTG GCTGCACCAT CTGTCTTCAT
                                                                          4.50
      CTTCCCGCCA TCTGATGAGC AGTTGAAATI T MAACTGCC TCTGTTGTGT GCCTGCTGAA
                                                                          540
      TAACTTOTAT COCAGAGAGG CCAAAGTAHA HIGGAAGGTG GATAACGCCC TCCAATCGGG
      TAACTCCCAG GAGAGTGTCA CAGAGCARAA 14 9 AAGGAC AGCACCTACA GCCTCAGCAG
                                                                          4.10
                                                                          560
35
      CACCCTGACG CTGAGCAAAG CAGACTACGA JAAACACAAA GTCTACGCCT GCGAAGTCAC
```

CCATCAGGGC CTGAGCTTGC CCGTCACAAA BA ECTTCAAC AGGGGAGAGT GTTAGTGAGA

TGATCCTCTA GA 732

(2) INFORMATION FOR SEQ ID NO:23:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10

- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
- 1 S Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp Leu Arg

 1 5 10 15

 Gly Ala Arg Cys Asp Ile Gln Met Thr

 20 25
- 20 (2) INFORMATION FOR SEQ ID NO:24:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 amino acids
 - (B) TYPE: amino acid
- 25 (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: protein
- 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Leu Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys

1 5 10

35 (2) INFORMATION FOR SEQ ID NO:25:

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(x): GE, UENCE DESCRIPTION: SEC ID NO:15:

10		SCATGGAGTT	TGGGTTGAGC	TGGGTTTTCC	TOGTGGCTCT	TTTAAGAGGT	7.1
	GTC:JAGTGT/	AGGTGCAGGT	GGTGGAGTGT	GGGGGAGGCC	TGGTCAGGCC	TGGCGGGTCC	
	CTAAGACTCT	CGTGTGCAGC	CTCTGGAACC	ACCCTCAGTG	GCTATACCAT	GCACTGGGTC	· · ·
	CGCCAGGCT	CAGGGAAGGG	GCTGGAGTGG	GTCTCATCCA	TTACTGGAGG	TAGCAACTTC	1 '
	ATAMACTACG	CAGACTCAGT	GAAGGGCCGA	TTCACCATCT	CCAGAGACAA	CGCCAAGAAC	• .
15	TOACTTTATC	TGCAAATGAA	CAGCCTGACA	GCCGAGGACA	CGGCTGTCTA	TTATTGTGUU	٠,
	ACCGCCCCTA	TAGCACCGCC	CTACTTTGAC	CACTGGGGCC	AGGGAACCCT	GGTCACCGTC	.
	TOOTCAGOOT	CCACCAAGGG	CCCATCGGTC	TTCCCCCTGG	CACCCTCCTC	CAAGAGCACC	· ±
	TCTGGGGGCA	CAGCGGCCCT	GGGCTGCCTG	GTCAAGGACT	ACTTCCCCGA	ACCGGTGACC	: <u>.</u> *
	GTGTCGTGGA	ACTCAGGCGC	CCTGACCAGC	GGCGTGCACA	CCTTCCCGGC	TGTCCTACAG	* ' '
20	TCCTCAGGAC	TCTACTCCCT	CAGCAGCGTG	GTGAČCGTGC	CCTCCAGCAG	CTTGGGCACC	: •
	CAGACCTACA	TCTGCAACGT	GAATCACAAG	CCCAGCAACA	CCAAGGTGGA	CAAGAAAGTT	-
	GAGCCCAAAT	CTTGTGACAA	AACTCACACA	TGCCCACCGT	GCCCAGCACC	TGAACTCETG	~ .
	GGGGGACCGT	CAGTCTTCCT	CTTCCCCCCA	AAACCCAAGG	ACACCCTCAT	GATCTCCCGG	
	ACCCCTGAGG	TCACATGCGT	GGTGGTGGAC	GTGAGCCACG	AAGACCCTGA	GGTCAAGTTC	0.
25	AACTGGTACG	TGGACGGCGT	GGAGGTGCAT	AATGCCAAGA	CAAAGCCGCG	GGAGGAGCAG	
	TACAACAGCA	CGTACCGGGT	GGTCAGCGTC	CTCACCGTCC	TGCACCAGGA	CTGGCTGAAT	
	GGCAAGGAGT	ACAAGTGCAA	GGTCTCCAAC	AAAGCCCTCC	CAGCCCCCAT	CGAGAAAACC	111 -
	ATCTCCAAAG	CCAAAGGGCA	GCCCCGAGAA	GCACAGGTGT	ACACCCTGCC	CCCATCCQDG	
	GATGAGCTGA	CCAAGAACCA	GGTCAGCCTĞ	ACCTGCCTGG	TCAAAGGCTT	CTATCCCAGI	1
30	GACATCGCCG	TGGAGTGGGA	GAGCAATGGG	CAGCCGGAGA	ACAACTACAA	GACCACGCCT	1 ~ .
	CCCGTGCTGG	ACTCCGACGG	CTCCTTCTTC	CTCTACAGCA	AGCTCACCGT	GGACAAGAGG	1 - 1.
	AGGTGGCAGC	AGGGGAACGT	CTTCTCATGC	TOUGTGATGC	ATGAGGCTCT	GCACAACCAC	1 15
	TACACGCAGA	AGAGCCTCTC	CCTGTCTCCG	GGTAAATGAT	AGATATC		1 11

35 (2) INFORMATION FOR SEQ ID NO:26:

```
(i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 7 amino acids
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
5
              (D) TOPOLOGY: linear
            (ii) MOLECULE TYPE: protein
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
10
      Ser Asn Phe Ile Asn Tyr Ala
       1
               (2) INFORMATION FOR SEQ ID NO:27:
15
            (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 1427 base pairs
              (B) TYPE: nucleic acid
              (C) STRANDEDNESS: single
20
              (D) TOPOLOGY: linear
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
25
      GAATTCGGTA CCATGGAGTT TGGGCTGAGC TGGGTTTTCC TCGTGGCTCT TTTAAGAGGT
                                                                            60
      GTCCAGTGTC AGGTGCAGCT GGTGGAGTCT GGGGGGAGGCC TGGTCAGGCC TGGCGGGTCC
                                                                            120
      CTAAGACTCT CGTGTGCAGC CTCTGGAACC ACCCTCAGTG GCTATACCAT GCACTGGGTC
                                                                            180
      CGCCAGGCTC CAGGGAAGGG GCTGGAGTGG GTCTCATCCA TTACTGGAGG TAGCAACTTC
                                                                            240
      ATACAATACT CAGACTCAGT GAAGGGCCGA TTCACCATCT CCAGAGACAA CGCCAAGAAC
                                                                            300
30
      TCACTTTATC TGCAAATGAA CAGCCTGACA GCCGAGGACA CGGCTGTCTA TTATTGTGCG
                                                                            360
      ACCGCCCTA TAGCACCGCC CTACTTTGAC CACTGGGGCC AGGGAACCCT GGTCACCGTC
                                                                            420
      TCCTCAGCCT CCACCAAGGG CCCATCGGTC TTCCCCCTGG CACCCTCCTC CAAGAGCACC
                                                                            480
      TCTGGGGGCA CAGCGGCCCT GGGCTGCCTG GTCAAGGACT ACTTCCCCGA ACCGGTGACC
                                                                            540
      GTGTCGTGGA ACTCAGGCGC CCTGACCAGC GGCGTGCACA CCTTCCCGGC TGTCCTACAG
                                                                            600
35
      TCCTCAGGAC TCTACTCCCT CAGCAGCGTG GTGACCGTGC CCTCCAGCAG CTTGGGCACC
                                                                            660
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CAGACCTACA TCTGCAACGT GAATCACAAG CCCAGCAACA CCAAGGTGGA CAAGAAAGTT

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                                  GATGAGCTGA CCAAGAACCA GOTCAGCCTG ACCTGCCTGG TCAAAGCCTT CTATCCCAGC
                                  JACATUBUCS TEGAGTEGGA GAGDAATGUS DAĞOOGGAGA ACAACTACAA GAMCACGOOT -
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                                 AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCA
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                                  TACACUCAGA AGAGCCTCTC CCTGTCTCCU GGTAAATGAT AGATATO
                                                                                                                                                                                                                                                                                                                                                                                                                                            1.12.00
                                                                                    (h) information for sec id no: 15:
15
                                                                    (i) SEQUENCE CHARACTERISTICS:
                                                                               (A) LENGTH: 7 amino acids
                                                                               (B) TYPE: amino acid
                                                                               (C) STRANDEDNESS: single
 20
                                                                            (D) TOPOLOGY: linear
                                                                     Wir MOLECULE TYPE: protein
                                                                     (x1) SEQUENCE DESCRIPTION: SEQ 1D NO:28:
 25
                                  Ser Asn Phe Ile Gln Tyr Ser
                                                                                       (2) INFORMATION FOR SEQ ID NO:29:
  30
                                                                      (i) SEQUENCE CHARACTERISTICS:
                                                                                  (A) LENGTH: 762 base bairs
                                                                                   (B) TYPE: nucleic acid
                                                                                   (C) STRANDEDNESS: single
  35
                                                                                  (D) TOPOLOGY: linear
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GAATTCCATG GACATGAGGG TCCCCGCTCA GCTCCTAGGG CTCCTGCTGC TCTGGCTCCG 60 5 AGGTGCCAGA TGTGACATCC AGATGACCCA GTCTCCATCC TCCCTGTCTG CATCTGTAGG 120 AGACAGAGTC ACCATCACTT GCCGGGCAAC TCAGAGTGTT AGTAACTTTT TAAATTGGTA 180 TCAGCAGAAG CCAGGGGAAG CCCCTACGCT CCTGATCTAT GATGCATCCA CTTCGCAAAG 240 TGGGGTCCCA TCAAGGTTCA GTGGCAGTGG ATCTGGGATG GATTTCAGTC TCACCATCAG 300 CAGTCTGCAG CCTGAAGATC TTGCAATGTA TTACTGTCAA GCGAGTATCA ATACCCCGCT 360 10 TTTCGGCGGA GGGACCAGAA TAGATATGAG ACGAACTGTG GCTGCACCAT CTGTCTTCAT 420 CTTCCCGCCA TCTGATGAGC AGTTGAAATC TGGAACTGCC TCTGTTGTGT GCCTGCTGAA 480 TAACTTCTAT CCCAGAGAGG CCAAAGTACA GTGGAAGGTG GATAACGCCC TCCAATCGGG 540 TAACTCCCAG GAGAGTGTCA CAGAGCAGGA CAGCAAGGAC AGCACCTACA GCCTCAGCAG 600 CACCCTGACG CTGAGCAAAG CAGACTACGA GAAACACAAA GTCTACGCCT GCGAAGTCAC 660 15 CCATCAGGGC CTGAGCTCGC CCGTCACAAA GAGCTTCAAC AGGGGAGAGT GTTAGTGAGA 720 TGATCCTCTA GATCTACGTA TGATCAGCCT CGACTGTGCC TT 762

(2) INFORMATION FOR SEQ ID NO:30:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

30 Ser Pro Val Thr Lys Ser Phe Thr Arg Gly Gln Cys

1 5 10

What is claimed is:

L. A reshaped human monoclonal antibody and functional fragments thereof, specifically reactive with an F protein epitope of Respiratory Syncytial Virus and capable of neutralizing infection by said virus selected from the group consisting of Hu19A, Hu19B, Hu19C and Hu19D.

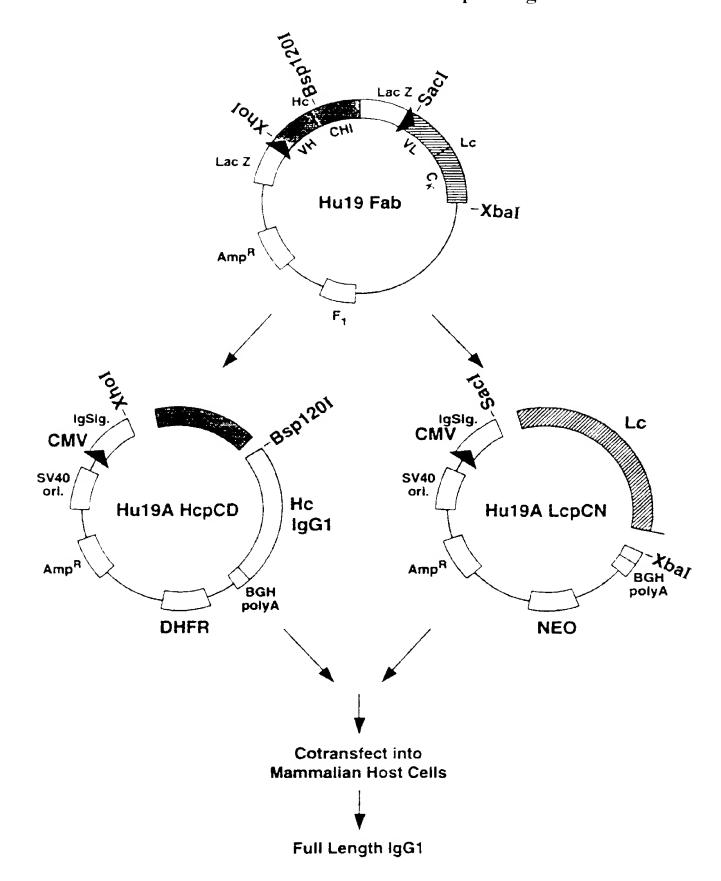
- 2. The monoclonal antibody or functional fragment thereof according to Claim 1 which comprises a light chain amino acid sequence of Figure 3 selected from Sequences 19A, 19B, 19C and 19D and/or a heavy chain amino acid sequence of Figure 2 selected from Sequences 19A, 19B, 19C and 19D.
- 3. The monoclonal antibody according to Claim 1 wherein said fragment is selected from the group consisting of Fv. Fab and F(ab')2.
- 4. An isolated nucleic acid molecule selected from the group consisting of:
- (a) a nucleic acid sequence encoding any of the human monoclonal antibodies and functional fragments thereof of claim 1;
 - (b) a nucleic acid complementary to any of the sequences in (a); and
- (c) a nucleic acid sequence of 18 or more nucleotides capable of hybridizing to (a) or (b) under stringent conditions.
- 5. A isolated nucleic acid molecule encoding a monoclonal antibody or functional fragment thereof according to Claim 1 having a nucleotide sequence of Figure 4.
- 6. A recombinant plasmid comprising a nucleic acid sequence of Claim
 4.
- 7. A recombinant plasmid comprising a nucleic acid sequence of claim 5.
- 8. A plasmid according to Claim 7 encoding a protein sequence of Figure 2 or 3.
 - 9. A host cell comprising the plasmid of Claim 8.

10. A process for the production of a human antibody specific for RSV comprising culturing the host cell of Claim 9 in a medium under suitable conditions of time, temperature and pH and recovering the antibody so produced.

- 11. A method of detecting RSV comprising contacting a source suspected of containing RSV with a diagnostically effective amount of the monoclonal antibody of Claim 1 and determining whether the monoclonal antibody binds to the source.
- 12. A method for providing passive immunotherapy to RSV disease in a human, comprising administering to the human an immunotherapeutically effective amount of the monoclonal antibody of Claim 1.
- 13. The method according to Claim 12 wherein the passive immunotherapy is provided prophylactically.
- 14. A pharmaceutical composition comprising at least one dose of an immunotherapeutically effective amount of the monoclonal antibody of Claim 1 in a pharmaceutically acceptable carrier.
- 15. A pharmaceutical composition comprising at least one dose of an immunotherapeutically effective amount of the monoclonal antibody of Claim 1 in combination with at least one additional monoclonal antibody.
- 16. The pharmaceutical composition according to Claim 15 wherein said additional monoclonal antibody is an anti-RSV antibody distinguished from the antibody of Claim 1 by virtue of being reactive with a different epitope of the RSV F protein antigen.

FIGURE 1

Conversion of Hu19 Fab to a Complete IgG1 mAb



2/31 Figure 2 Comparison of the Heavy Chain Amino Acid Sequences of various Hu19 mAbs

GL Dp58:	EVQL VESGGGLVQPGGSLRLSC	AASGFTFS	30
19A:	MGWSCIILFLVATATGVHSLER	T-L-	
19B:	-EFGLSWVLLRQCQVQL V		
19C:			
19D:			
	CDR1 CDR2		
GL Dp58	SYEMNWVRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISR	DNAKNSLY	80
19A	G-T-HS-TGGSNF- <u>N-S</u>		
19B			
19C:	A		
19D:	Q-S		
	CDR3		
GL: Dp58	LQMNSLRAEDTAVYYCAR 98	(SEQ ID N	NO: 4)
19A:	TTAPIAPPYFDHWGQGTLVTVSS	(SEQ ID N	NO: 5)
19 B :		(SEQ ID N	10: 6)
19C:		(SEQ ID N	NO: 7)
19D:		(SEQ ID N	10: 8)

3/31 Figure 3 Comparison of the Light Chain Amino Acid Sequences of various Hu19 mAbs

A. Leader and Variable

			CURI	
GL Dpk9	DŢ.	ÇMTÇBPUSI.GAĞVGDE	UTITORASQSIS	à e
19A=	MGWSCIILFLVATATGVHS	Eb	TV-	28
19B.C.D	MRVPAQLLGLLLLWLRGARCOI	QM ∞		
	co	R.1		
		-		
GL Dpk9	syln wyqqkpgkapklliy aas	slqs gvpsrf,ggsgs	ATDETLI ISSLOP	<u> </u>
19A.	NFLN E TD	TS	-MS	7 9
19B,C.D.				
	CDR3			
GL Dpk9	EDFATYYC *		(SEQ ID	NC: 9)
19A:	L-M QASINTPL FGGGTR	IDMPP 105	(SEQ ID	NG: 10)
19B.C.D			(SEQ ID	NO: 11:
B. Con	stant Region (Cκ)			
	TVAAPSVFIFPPSDEQLKSGTA	@VVCUUNNFYPREAK	VQWKVDNALQSGN	
19A,B:			- · · · · · · · · · · · · · · · · · · ·	
			······································	
	SQESVTEQDSKDSTYSLSSTI.T			
19A,B			1	
11 1000	(27)			
Hu-κ,19C,D				
19A.B	(SEQ ID	[. :: » :		

4/31 Figure 4

Figure 4A-- DNA sequence of the plasmid Hu19AHcpcd

1	gacgtegeggeegetetaggeetecaaaaageeteeteaetaettetgg	50
51	aatagctcagaggccgaggcctcggcctctgcataaataa	100
101	tagtcagccatgcatggggggagaatgggcggaactgggcggagttagg	150
151	ggcgggatgggcggggctatggttgctgactaattgag	200
201	atgcatgctttgcatacttctgcctgctggggagcctggggactttccac	250
251	acctggttgctgactaattgagatgcatgctttgcatacttctgcctgc	300
301	ggggagcctggggactttccacaccctaactgacacacattccacagaat	350
351	taattcccggggatcgatccgtcgacgtacgactagttattaatagtaat	400
401	caattacggggtcattagttcatagcccatatatggagttccgcgttaca	450
451	taacttacggtaaatggcccgcctggctgaccgcccaacgacccccgccc	500
501	attgacgtcaataatgacgtatgttcccatagtaacgccaatagggactt	550
551	tccattgacgtcaatgggtggactatttacggtaaactgcccacttggca	600
601	gtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatga	650
651	cggtaaatggcccgcctggcattatgcccagtacatgaccttatgggact	700
701	ttcctacttggcagtacatctacgtattagtcatcgctattaccatggtg	750
751	atgcggttttggcagtacatcaatgggcgtggatagcggtttgactcacg	800

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9:1	tigggtängtgaanogtnagatngontggagangnnatngaattntgagna	Let
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1151	aggeetggteaggeetggegggtenetaagaetetegtgtgeageetetg	2.2
1151	gaaccaccctcagtggctataccatgcactgggtccgccaggctccaggg	1204
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1301	agaactcactttatctgcaaatgaacagcctgacagccgaggacacggct	1 7 5
1351	gtotattattgtgogacogoccotaragcácogocotactttgaccactg	140
1401	gggccagggaaccotggtcaccgtctcctcagcotccaccaagggcccat	145
1451	caatetteecetaacaeetestenaadadeacetetadadacadeu	

1501	gccctgggctgcctggtcaaggactacttccccgaaccggtgaccgtgtc	1550
1551	gtggaactcaggcgcctgaccagcggcgtgcacaccttcccggctgtcc	1600
1601	tacagtcctcaggactctactccctcagcagcgtggtgactgtgccctcc	1650
1651	agcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccag	1700
1701	caacaccaaggtggacaagaagttgagcccaaatcttgtgacaaaactc	1750
1751	acacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtc	1800
1801	ttectetteccecaaaacccaaggacaccetcatgateteceggacece	1850
1851	tgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtca	1900
1901	agttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag	1950
1951	ccgcgggaggagcagtacaacagcacgtaccgggtggtcagcgtcctcac	2000
2001	cgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtct	2050
2051	ccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaa	2100
2101	. gggcagccccgagaaccacaggtgtacaccctgcccccatcccgggatga	2150
2151	gctgaccaagaaccaggtcagcctgacctggctggtcaaaggcttctatc	2200
2201	ccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaac	2250
2251	tacaagaccacgcctcccgtgctggactccgacggctccttcttcctcta	2300
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3101	gadagotoagggotgogatttogögosaäaottgaoggoaatootagogt	3157
3151	gaaggetggtaggattttateneegetgeeateatggttegaeeattgaa	3200

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3551	aggaagccatgaatcaaccaggccaccttagactctttgtgacaaggatc	3600
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3651	atataaacttctcccagaatacccaggcgtcctctctgaggtccaggagg	3700
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3951	cgcattgtctgagtaggtgtcattctattctggggggtggggtggggcag	4000
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4451	atcaggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaagg	4550
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4601	cccgacaggactataaagataccaggcgtttccccctggaagetecctcg	4650
4651	tgogototootgttoogaccotycogottacoggatacctgtoogcottt	4 73)
4701	ctcccttcgggaagcgtggcgctttctcaatgctcacgctgtaggtatct	4700
4751	cagtteggtgtaggtegttegeteeaagetgggetgtgtgeaegaaceee	4800
4801	cogttcagecogacogotgogocttatooggtaactatogtottgagtoo	4850
4851	aaccoggtaagacangacttatogccactggcagcagccactggtaacaq	4 900

901	gactageagagegaggtaegtaggeggtgetaeagagttettgaagtggt	4950
1951	ggcctaactacggctacactagaaggacagtatttggtatctgcgctctg	5000
5001	ctgaagccagttaccttcggaaaaagagttggtagctcttgatccggcaa	5050
5051	acaaaccaccgctggtagcggtggttttttttgtttgcaagcagcagatta	5100
5101	cgcgcagaaaaaaggatctcaagaagatcctttgatcttttctacgggg	5150
5151	tctgacgctcagtggaacgaaaactcacgttaagggattttggtcatgag	5200
5201	attatcaaaaaggatcttcacctagatccttttaaattaaaaatgaagtt	5250
5251	ttaaatcaatctaaagtatatatgagtaaacttggtctgacagttaccaa	5300
5301	tgcttaatcagtgaggcacctatctcagcgatctgtctatttcgttcatc	5350
5351	catagttgcctgactccccgtcgtgtagataactacgatacgggagggct	5400
5401	taccatctggccccagtgctgcaatgataccgcgagacccacgctcaccg	5450
5451	gctccagatttatcagcaataaaccagccagccggaagggccgagcgcag	5500 ·
5501	aagtggtcctgcaactttatccgcctccatccagtctattaattgttgcc	5550
5551	gggaagctagagtaagttcgccagttaatagtttgcgcaacgttgtt	5600
5601	gccattgctacaggcatcgtggtgtcacgctcgtcgtttggtatggcttc	5650
5651	attcagctccggttcccaacgatcaaggcgagttacatgatcccccatgt	5700
5701	tgtgcaaaaagcggttagctccttcggtcctccgatcgttgtcagaagt	5750

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52 5 1	ctcatqaqcggatacatatttgäatqtatttaqaaaaataaacaaatagg	6.1.)
6251	ggtteegegeacattteeeegaaaagtgeeacet 6284 (SEQ ID No	:14

Figure 4B-- DNA sequence of the plasmid Hu19ALcpcn:

_	guegeegeegeeeeeuuduuugeeeeeuucueegg	30
51	aatagctcagaggccgaggcctcggcctctgcataaataa	100
101	tagtcagccatgcatgggcggagatgggcggaactgggcggagttagg	150
151	ggcgggatgggcggagttaggggggactatggttgctgactaattgag	200
201	atgcatgctttgcatacttctgcctgctggggagcctggggactttccac	250
251	acctggttgctgactaattgagatgcatgctttgcatacttctgcctgc	300
301	ggggagcctggggactttccacaccctaactgacacacattccacagaat	350
351	taattcccggggatcgatccgtcgacgtacgactagttattaatagtaat	400
401	caattacggggtcattagttcatagcccatatatggagttccgcgttaca	450
451	taacttacggtaaatggcccgcctggctgaccgcccaacgacccccgccc	500
501	attgacgtcaataatgacgtatgttcccatagtaacgccaatagggactt	550
551	tccattgacgtcaatgggtggactatttacggtaaactgcccacttggca	600
601	gtacatcaagtgtatcatatgccaagtacgcccctattgacgtcaatga	650
651	cggtaaatggcccgcctggcattatgcccagtacatgaccttatgggact	700
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· - ·	ាក់ប្រើប្រទេសប្រធានិងសហរបស់ងំបាន។ ១ សេចបញ្ជាត់កាំងសំពុំក្លាន។ ១ <u>បង្គេប់ប</u> ត្តការាងមួយម	
1.35.1	nanaggaectbacca <u>t</u> gggatggagnngtatcatchtchtchtchtggtagca M G W C P I I E F L V A Leader start	.úz
1091	acagetaeaggtgteeaethngaqbrhaeneagfcineatectenetyfe T A T G V H S <u>E D T</u> Q S P - (SEQ ID Processed M-term.	
1151	ugsatetqtaqqaqacagagtsacsatcaettqesqqqqaactcagagtq	1187
1151	ttagtaaetttttaaattggtatragcagaagccaggggaagccentacg	1251
1201	otootgatotatgatgoatooau;toqoaaagtggggtoocatcaaggtt	1
. 11. 11.	cagtggcagtggatctgggatttcagtctcaccatcagcagtctqc	13.0
1301	ageotgaagatottgcaatgtathueffteaaggagtatcaatacceng	1353
1351	etttteggeggagggaeeagaatumanutgagakgaaetgtggetgeaee	14%)
1401	atotgtottoatottooogonar makkaagoagttgaaatotggaactg	1451
1451	cetetgttgtgtgcetgetgaaruu ",ctuteceagagggceaaagta	1550
1501	cagtggaaggtggataacgcom, man, migtaactcccaggagagtgt	1550

1551	cacagagcaggacaggacagcacctacagcctcagcagcaccctga	1600
1601	cgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtc	1650
1651	acccatcagggcctgagcttgcccgtcacaaagagcttcaacaggggaga L P V T K S F N R G E	1700
	Xba I	
1701	gtgttagtgagatgatcctctagagtcatctacgtatgatcagcctcgac C * end of light chain (SEQ ID NO: 19)	1750
1751	tgtgccttctagttgccagccatctgttgtttgcccctcccccgtgcctt	1800
1801	ccttgaccctggaaggtgccactcccactgtcctttcctaataaaatgag	1850
1851	gaaattgcatcgcattgtctgagtaggtgtcattctattctggggggtgg	1900
1901	ggtggggcaggacagcaagggggggggggttgggaagacaatagcaggcatg	1950
1951	ctggggatgcggtgggctctatggaaccagctggggctcgacagctcgag	2000
2001	ctagctttgcttctcaatttcttatttgcataatgagaaaaaaaggaaaa	2050
2051	ttaattttaacaccaattcagtagttgattgagcaaatgcgttgccaaaa	2100
2101	aggatgctttagagacagtgttctctgcacagataaggacaaacattatt	2150
2151	cagagggagtacccagagctgagactcctaagccagtgagtg	2200
2201	ttctagggagaaatatgcttgtcatcaccgaagcctgattccgtagagcc	2250
2251	acaccttggtaagggcaatctgctcacacaggatagaggggaggga	2300

a de Servador	ा भ्युवयुर्भयुक्षप्रकारकारकाक्ष्यव्या प्रक्षप्राध्य स्वया एक्ष्या एक्ष्या एक्ष्या ।	10
•	Ethidaeatadthdtdt เป็นผลงางประเทศ ประชาสุด การสดงาสปัญหาโปลและส	.
J40.1	adatgdattgcacgcagqtrotccddccdcttgggtggagaggctatrog	~ ;
2451	gctatgactgggcacaacagacaatcggctgctctgatgccgccgtgttc	155 ···
2501	cggctqtcagcqcaggggcgcccggttctttttgtcaagaccgacctgtc	255
1551	cggtgccctgaatgaactgcaggacgaggcagcggggctatcgtggctgg	2600
2601	ccacgacgggcgttccttgcgcagctgtgctcgacgttgtcactgaagcg	2650
2651	ggaagggactggctattgggcgaagtgccggggcaggatctcctgtc	2700
2701	ateteaeettgeteetgeegagaaagtateeateatggetgatgeaatge	2750
2751	ggeggetgeataegettgateeggetaeetgeeeattegaeeaeeaageg	2800
2801	aaacatcgcatcgagcgagcacgtactcggatggaagccggtcttgtcga	2850
2851	tcaggatgatctggacgaagagcatcaggggctcgcgccagccgaactgt	2900
2901	tegecaggeteaaggegegeatgeeegaeggegaggatetegtegtgaee	3027
2951	catggcgatgcctgcttgccgaatatcatggtggaaaatggccgcttttc	3:100
3001	tggattcatcgactgtggccggctgggtgtggggggaccgctatcaggaca	3050
3051	tagegttggetaceegtgatattgetgaagagettggeggegaatggget	:100

3101	gaccgcttcctcgtgctttacggtatcgccgctcccgattcgcagcgcat	3150
3151	. cgccttctatcgccttcttgacgagttcttctgagcgggactctggggtt	3200
3201	cgaaatgaccgaccaagcgacgcccaacctgccatcacgagatttcgatt	3250
3251	ccaccgccgccttctatgaaaggttgggcttcggaatcgttttccgggac	3300
3301	gccggctggatgatcctccagcgcggggatctcatgctggagttcttcgc	3350
3351	ccaccccaacttgtttattgcagcttataatggttacaaataaagcaata	3400
3401	gcatcacaaatttcacaaataaagcatttttttcactgcattctagttgt	3450
3451	ggtttgtccaaactcatcaatgtatcttatcatgtctggatcgcggccgc	3500
3501	gatcccgtcgagagcttggcgtaatcatggtcatagctgtttcctgtgtg	3550
3551	aaattgttatccgctcacaattccacacaacatacgagccggaagcataa	3600
3601	agtgtaaagcctggggtgcctaatgagtgagctaactcacattaattgcg	3650
3651	ttgcgctcactgccgctttccagtcgggaaacctgtcgtgccagctgca	3700
3701	ttaatgaatcggccaacgcgcggggagagggcggtttgcgtattgggcgct	3750
3751	cttccgcttcctcgctcactgactcgctgcgctcggtcgttcggctgcgg	3800
3801	cgagcggtatcagctcactcaaaggcggtaatacggttatccacagaatc	3850
3851	aggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggcca	3900
3901	adaaccataaaaadaccacattactagcatttttccatagactccaccc	3950

:05i	entadopadraticándadasecadequitodagtodadadatud Dayda en e	4.0
· · · ·	ាធិតិតផ្លើងការផ្ទៃតែស្ថិតស្ថិតស្ថិតផ្លាប់ពីប្រាក់ពីស្ថិតស្វាធិតិ និងស្ថិតស្ថិត បានប្រាក់ បានបានបានបានបានបានបានបានបានបានប្រាក់ បានប្រាក់ បានប្រាក់ បានប្រាក់ បានប្រាក់ បានប្រាក់ បានប្រាក់ បានប្រាក់ បានប្រាក់ បានបានប្រាក់ បានប្រាក់ បានប្រសាធិតិ បានប្រាក់ បានប	4 4.
4001	Weterestattesquessimesanttuseggatusetgtensentttetg	41,1
4101	coutogggaagogtggcgctttctcaatqctcacqctgtaqgtatctcac	4150
4151	ttoggtgtaggtogttogötocaaqotgggotgtgtgcacgaaccccccg	4 200
4201	tteageeegacegetgegeettateeggtaactategtettgayteeaac	4250
4251	ccqgtaagacacgacttatcgccactggcagcagccactggtaacaggat	430
4301	tagcagagcgaggtatgtaggcggtgctacagagttcttgaagtggtggc	4350
4351	ctaactacggctacactagaaggacagtatttggtatctgcgctctgctg	4400
4401	aagccagttaccttcggaaaaagagttggtagctcttgatccggcaaaca	4450
4451	aaccaccgctggtagcggttgtttttttgtttgcaagcagcagattacgc	4500
4501	gcagaaaaaaaggateteaagaagateetttgatettttetaeggggtet	4550
4551	gacgctcagtggaacgaaaactcacgttaagggattttggtcatgagatt	4600
4601	atcaaaaaggatcttcacctagatccttttaaattaaaaatgaagtttta	4650
4651	aatcaatctaaagtatatatgagtaaacttggtctgacagttaccaatgc	470°
4701	ttaatcagtgaggcacctatctcagcgatctgtctatttcgttcatccat	4 750
4751	agttgcctgactccccgtcgtgtagataactacgatacgggagggcttac	4300

4801	catctggccccagtgctgcaatgataccgcgagacccacgctcaccggct	4850
4851	. ccagatttatcagcaataaaccagccagccggaagggccgagcgcagaag	4900
4901	tggtcctgcaactttatccgcctccatccagtctattaattgttgccggg	4950
4951	aagctagagtaagttcgccagttaatagtttgcgcaacgttgttgcc	5000
5001	attgctacaggcatcgtggtgtcacgctcgtcgtttggtatggcttcatt	5050
5051	cagctccggttcccaacgatcaaggcgagttacatgatcccccatgttgt	5100
5101	gcaaaaaagcggttagctccttcggtcctccgatcgttgtcagaagtaag	5150
5151	ttggccgcagtgttatcactcatggttatggcagcactgcataattctct	5200
5201	tactgtcatgccatccgtaagatgcttttctgtgactggtgagtactcaa	5250
5251	ccaagtcattctgagaatagtgtatgcggcgaccgagttgctcttgcccg	5300
5301	gcgtcaatacgggataataccgcgccacatagcagaactttaaaagtgct	5350
5351	catcattggaaaacgttcttcggggcgaaaactctcaaggatcttaccgc	5400
5401	tgttgagatccagttcgatgtaacccactcgtgcacccaactgatcttca	5450
5451	gcatcttttactttcaccagcgtttctgggtgagcaaaacaggaaggca	5500
5501	aaatgccgcaaaaaagggaataagggcgacacggaaatgttgaatactca	5550
5551	tactcttcctttttcaatattattgaagcatttatcagggttattgtctc	5600
5601	atgagcggatacatatttgaatgtatttagaaaaataaacaaataggggt	5650
5651	tccgcgcacatttccccgaaaagtgccacct 5681 (SEQ ID NO: 17)

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Figure 4C. Heavy chain coding sequence in the plasmid Hu19BHeped

	Foo El	
	<u>iaat*:ggtacc</u>	105
1001	<u>atggagtttgggctgagctgggttttcctcgtggctcttttaagaggtgt</u>	1050
	M E F G L S W V F L V A L L F G V Leader start	
1051	Ccagtgtcaggtgcagctggtggaggtctgggggagggcttggtcaggcctg Q C Q V Q L V - (SEQ ID NO: 21) Processed N-term	1100
1101	gogggtocctaagactotogtgtgcagoctotggaaccaccotcagtggc	1150
1151	tataccatgcactgggtccgccaggctccagggaaggggctggagtgggt	1296
1201	ctcatccattactggaggtagcaacttcataaactactcagactcagtga	1250
1251	agggccgattcaccatctccagagacaacgccaagaactcactttatctg	1300
1301	caaatgaacagcctgacagccgaggacacggctgtctattattgtgcgac	1350
1351	cgcccctatagcaccgccctactttgaccactggggccagggaaccctgg	1400
1401	tcaccgtctcctcagcctccaccaagggcccatcggtcttccccctggca	1450
1451	ccctcctccaagagcacctctgggggcacagcggccctgggctgcctggt	1500
1501	caaggactacttccccgaaccggtgaccgtgtcgtggaactcaggcgccc	1550
1551	tgaccagoggogtgcacacottcccggntgtcctacagtcctcaggactc	1600

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1601	tactccctcagcagcgtggtgaccgtgccctccagcagcttgggcaccca	1650
1651	. gacctacatctgcaacgtgaatcacaaggcccagcaacaccaaggtggaca	1700
1701	agaaagttgagcccaaatcttgtgacaaaactcacacatgcccaccgtgc	1750
1751	ccagcacctgaactcctggggggaccgtcagtcttcctcttcccccaaa	1800
1801	acccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtgg	1850
1851	tggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtg	1900
1901	gacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagta	1950
1951	caacagcacgtaccgggtggtcagcgtcctcaccgtcctgcaccaggact	2000
2001	ggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctccca	2050
2051	gcccccatcgagaaaccatctccaaagccaaagggcagccccgagaacc	2100
2101	acaggtgtacaccctgccccatcccgggatgagctgaccaagaaccagg	2150
2151	tcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtg	2200
2201	gagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcc	2250
2251	cgtgctggactccgacggctccttcttcctctacagcaagctcaccgtgg	2300
2301	acaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcat	2350
2351	gaggetetgeacaaceactacaegeagaagageeteteeetgteteeggg S P G	2400

2401 taaatgatagatatc - (SEQ ID NO:20)

K \star end of heavy chain

Figure 4D Light chain coding sequence in the plasmid Hu19BLepen

	Eco FI	
	mant meatgga	1000
1051	catgagggtccccgctcagctcctagggctcctgctctggctccgag	1.750
	M P V P A Q L L G L L L W L R	
	Leader start	
1051	gtgccagatgtgacatccagatgacccagtctccatcctccctqtctgca	1100
	G A R C D I Q M T - (SEQ ID NO: 23)	
	Processed N-term	
1101	totgtaggagacagägtcaccatcacttgccgggcaactcagagtgttag	1150
1151	taactttttaaattggtatcagcagaagccaggggaagcccctacgctcc	1200
1201	tgatetatgatgcarccacttegcaaagtggggteecatcaaggtteagt	1250
1251	ggcagtggatctgggatttcagtctcaccatcagcagtctgcagcc	1300
1301	tgaagatettgeaatgtattaetgteaagegagtateaataeeeegettt	1350
1351	tcggcggagggaccagaatagatatgagacgaactgtggctgcaccatct	1400
1401	gtottoatottocogocatotgatgagcagttgaaatotggaactgcoto	1450
1451	tgttg stgcctgctgaataacttctatcccagagaggccaaagtacagt	1500
1501	ggaaggtggataacgccctccaatcgggtaactcccaggagagtgtcaca	1550
1551	gagcaggacagcaaggacagcatacagcctcaqcaqcaccctgacgct	1600
1601	gagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcaccc	1650

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1651 atcagggcctgagcttgcccgtcacaaagagcttcaacaggggagagtgt 1700

L P V T K S F N R G E C (SEQ NO: 24)

Xba I

1701 <u>tagtgagatgatcctctaga</u> (SEQ ID NO: 22)

* end of light chain

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Figure 4E Heavy chain coding sequence in the plasmid Hu19CHeped

	\mathbb{R}^{+} - \mathbb{R}^{1}	
	<u>daattoggtacc</u>	
15%1	atggagtttgggctgagctgggttttcctcgtggctcttttaagaggtgt M E F G L S W V F L V A L L R G V	105)
1311	ccagtgtcaggtgcagctggtggagtctqggggaggcctqgtcaggcctg Q C Q V Q L V - (SEQ ID NO: 21) Processed N-term	1100
1101	grgggtccctaagactctcgtgtgcagcctctggaaccaccctcagtggc	1150
1151	tataccatgcactgggtccgccaggctccagggaaggggctggagtgggt	1200
1201	ctcatccattactggaggtagcaacttcataaactac \underline{g} cagactcagtga S N F I N Y \underline{A} - (SEQ ID NO	1250 : 26)
1251	agggeegatteaccateteeagagacaacgeeaagaaeteactttatetg	1300
1301	caaatgaacagcctgacagccgaggacacggctgtctattattgtgcgac	1350
1351	cgccctatagcaccgcctactttgacqactggggccagggaaccctgg	1400
1401	tcaccgtctcctcagcctccaccaaggggccatcggtcttccccctggca	2450
1451	ccctcctccaagagcacctctgggggcacagcggccctgggctgcctggt	1500
1501	caaggactacttccccgaaccggtgaccgtqtcgtggaactcaggcgccc	1550
1551	tgaddagnggngtgdanaddtthdnigdtyteetadagtdotdaggadtd	1600
1601	tactccctcagcagcgtggtgaccqtdccctccagcagcttgggcaccca	165(

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1651	gacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggaca	1700
1701	agaaagttgagcccaaatcttgtgacaaaactcacacatgcccaccgtgc	1750
1751	ccagcacctgaactcctggggggaccgtcagtcttcctcttcccccaaa	1800
1801	acccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtgg	1850
1851	tggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtg	1900
1901	gacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagta	1950
1951	caacagcacgtaccgggtggtcagcgtcctcaccgtcctgcaccaggact	2000
2001	ggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctccca	2050
2051	gccccatcgagaaaccatctccaaagccaaagggcagccccgagaacc	2100
2101	acaggtgtacaccctgccccatcccgggatgagctgaccaagaaccagg	2150
2151	tcagectgacetgcetggtcaaaggettetateceagegacategeegtg	2200
2201	gagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcc	2250
2251	cgtgctggactccgacggctccttcttcctctacagcaagctcaccgtgg	2300
2301	acaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcat	2350
2351	gaggetetgeacaaceactacaegeagaagageeteteeetgteteeggg S P G	2400

2401 taaa<u>tga</u>tagatatc - (SEQ ID NO: 25)

K * end of heavy chain

Figure 4F Heavy chain coding sequence in the plasmid Hu19DHcpcd

	Eco El	
* (1)	ida: : cggtacc	
1050	atggagtttgggctgagctgggttttcctcgtggctcttttaagaggtgt M E F G L S W V F L V A L L R G V	1001
1100	ccagtgtcaggtgcagctggtggaqtctgggggggggggcctggtcaggcctg	1051
	Q C Q V Q L V - (SEQ ID NO: 21) Processed N-term	100
1150	gegggtecetaagaetetegtgtgeageetetggaaeeaeeeteagtgge	1101
1200	tataccatgcactgggtccgccaggctccagggaaggggctggagtgggt	1151
1250	ctcatccattactggaggtagcaacttcatacaaattcagactcagtga	1201
130%	S N F I Q Y S - (SEQ ID N agggeogatteaceatetecagagacaaegeeaagaacteaetttatetg	1251
1350	caaatgaacagcctgacagccgaggacacggctgtctattattgtgcgac	1301
1460	cgcccctatagcaccgccctactttgaccactggggccagggaaccctgg	1351
1450	teacegtetecteageetecaceaagggeeeateggtettecceetggea	1401
1500	ceeteeteeaagageaeetetgggggeaeageggeeetgggetgeetggt	1451
155%	caaggactacttccccgaaccggtgaccgtgtcgtggaactcaggcgccc	1501
1600		1551
1651	tactccctcaqcaqcqtqqtqaccqtqccctccaqcaqcttqggcaccca	1601

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1651	gacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggaca	1700
1701	. agaaagttgagcccaaatcttgtgacaaaactcacacatgcccaccgtgc	1750
1751	ccagcacctgaactcctggggggaccgtcagtcttcctcttcccccaaa	1800
1801	acccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtgg	1850
1851	tggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtg	1900
1901	gacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagta	1950
1951	caacagcacgtaccgggtggtcagcgtcctcaccgtcctgcaccaggact	2000
2001	ggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctccca	2050
2051	gcccccatcgagaaaccatctccaaagccaaagggcagccccgagaacc	2100
2101	acaggtgtacaccctgccccatcccgggatgagctgaccaagaaccagg	2150
2151	tcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtg	2200
2201	gagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcc	2250
2251	cgtgctggactccgacggctccttcttcctctacagcaagctcaccgtgg	2300
2301	acaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcat	2350
2351	gaggctctgcacaaccactacacgcagaagagcctctccctgtctccggg	2400
2401	tanatantagatata - (SEO ID NO. 27)	

2401 taaa<u>tga</u>tagatatc - (SEQ ID NO: 27)

K * end of heavy chain

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Figure 4G Light chain coding sequence in the plasmid H19CLepen

	Etw. Fl	
	<u> Taurincatgga</u>	• 1,
1001	<pre>catgagggtccccgctcagctcctagggctcctgctctgctccgag M R V P A Q L L G L L L L W L R Leader start</pre>	195.
1051	<pre>gtgccagatgtgacatccagatgacccagtctccatcctccctqtttgca G A R C D I Q M T - (SEQ ID NO: 13)</pre>	1100
1101	tetgtaggagacagagteaccateacttgccgggcaactcagagtgttag	1150
1151	taactttttaaattggtatcagcagaagccaggggaagcccctacgctcc	1200
1201	tgatotatgatgcatocacttogcaaagtggggtcocatoaaggttcagt	1250
1251	ggeagtggatetgggattteagteteaceateageagtetgeagee	1300
1301	tgaagatettgeaatgtattaetgteaagegagtateaataeeeegettt	1350
1351	toggoggagggaccagaatagatatgagacgaactgtggctgcaccatot	1400
1401	gtottoatottocogocatotgatqagoagttgaaatotggaactgcoto	1450
1451	tgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagt	1500
1501	ggaaggtggataacgccctccaatcgggtaactcccaggagagtgtcaca	1550
1551	gagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgct	1600

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1601	gagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcaccc	1650
------	--	------

1651 atcagggcctgagctgcgccgtcacaaagagcttcaacaggggagagtgt 1700

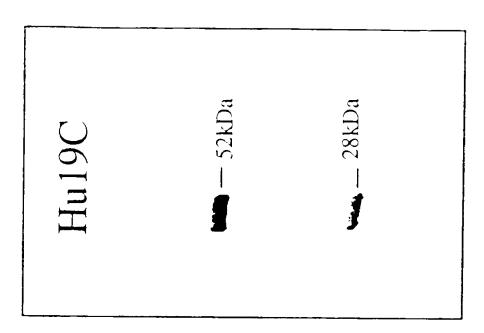
 \underline{S} P V T K S F T R G Q C (SEQ NO: 30)

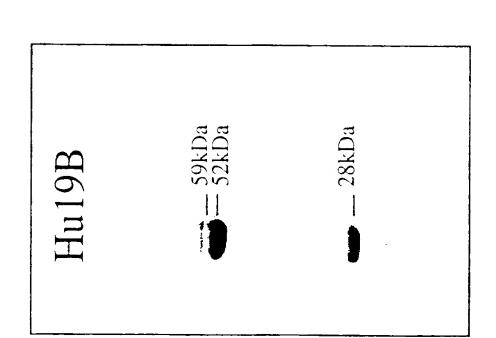
Xba I

1701 <u>tag</u>tgagatgatcc<u>tctaga</u>tctacgtatgatcagcctcgactgtgcctt -(SEQ NO: 29)

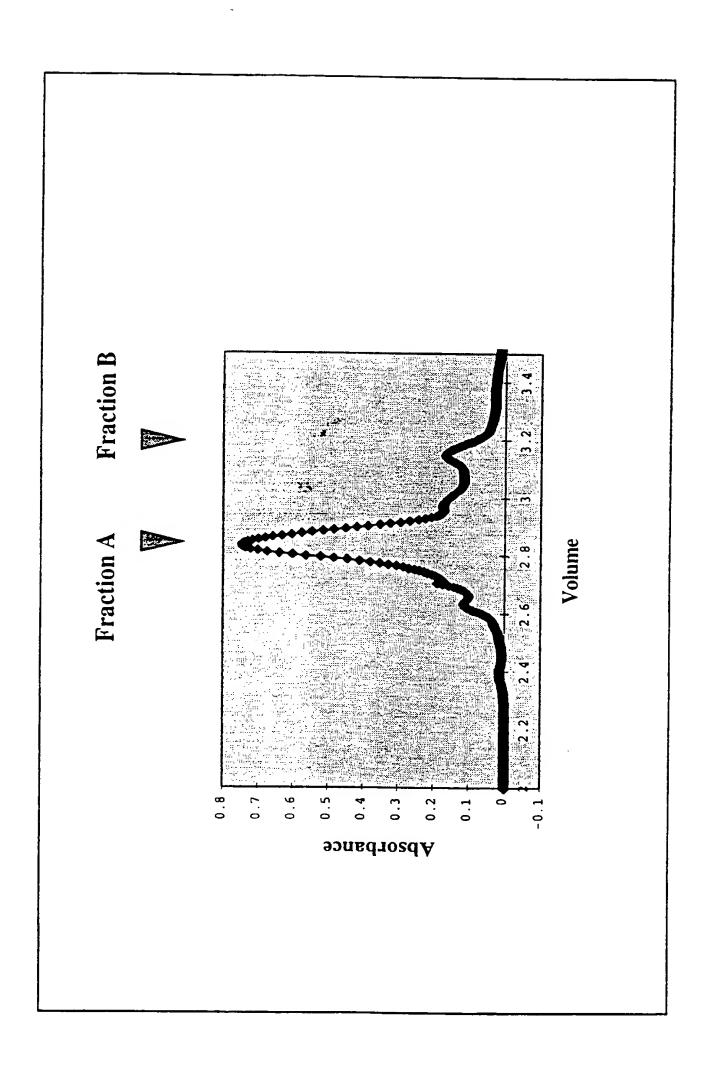
* end of light chain

RESPECTIVELY UNDER REDUCING CONDITIONS Fig. 5. COOMASSIE STAINED SDS-PAGE GEL ANALYSIS OF 10UG HU19B AND HU19C



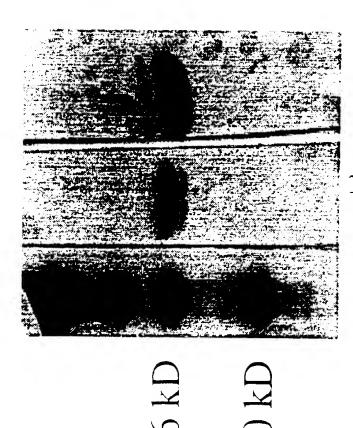


3. 6. SEPARATION OF HU19B GLYCOVARIANTS BY ANION EXCHANGE CHROMATOGRAPHY



BYST-000 140 601070/41

Fig. 7. SDS-PAGE of 3lycosylation Variant of Hu19B



Glycovariant

Mormal FAb

INTERNATIONAL SEARCH REPORT

International application No. PCT/US97/19203

A. CLASSIFICATION OF SUBJECT MATTER IPC(6): A61K 39/395 US CL: 530/387.3, 388.3; 424/159.1, 133.1 According to International Patent Classification (IPC) or to both national classification and IPC								
B. FIELDS SEARCHED								
Minimum documentation searched (classification system followed by classification symbols)								
U.S. : 530/387.3, 388.3; 424/159.1, 133.1								
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched								
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) APS. MEDLINE, DIALOG search terms: respiratory syncytial virus, reshaped human monoclonal antibody								
C. DOCUMENTS CONSIDERED TO BE RELEVANT								
								
Category*	Citation of document, with indication, where a	ppropriate, of the relev	ant passages	Relevant to claim No.				
A	TEMPTEST et al Reshaping a human monoclonal antibody to inhibit human respiratory syncytial virus infection in vivo. Bio/Technology. March 1994. Vol 9. Pages 266-271. See entire document.							
A	GROOTHIUS et al. Prophylactic a syncytial virus immune globulin to childern. The New England Journal 1993. Volume 329, Number 21, p document.	high-risk infants of Medicine. 18	and young November	1-11 and 14-16				
X Furth	ner documents are listed in the continuation of Box C	C. See paten	t family annex.					
"A" doc	ecial categories of cited documents: cument defining the general state of the art which is not considered	date and not in	published after the inte- conflict with the appl theory underlying the	ernational filing date or priority ication but cited to understand invention				
	be of particular relevance	"X" document of p	articular relevance; the	o claimed invention cannot be				
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other		considered novel or cannot be considered to involve an inventive step when the document is taken alone						
"O" document referring to an oral disclosure, use, exhibition or other means		"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination						
*P° document published prior to the international filing date but later than *& document member of the same patent family the priority date claimed								
	actual completion of the international search ARY 1998	Date of mailing of the international search report 2 3 FEB 1998						
Commission Box PCT	nailing address of the ISA/US ner of Patents and Trademarks n, D.C. 20231	JULIE E. REEVES						
Facsimile N		Telephone No. (7	03) 308-0196	•				

INTERNATIONAL SEARCH REPORT

International application No. PCT/US97/19203

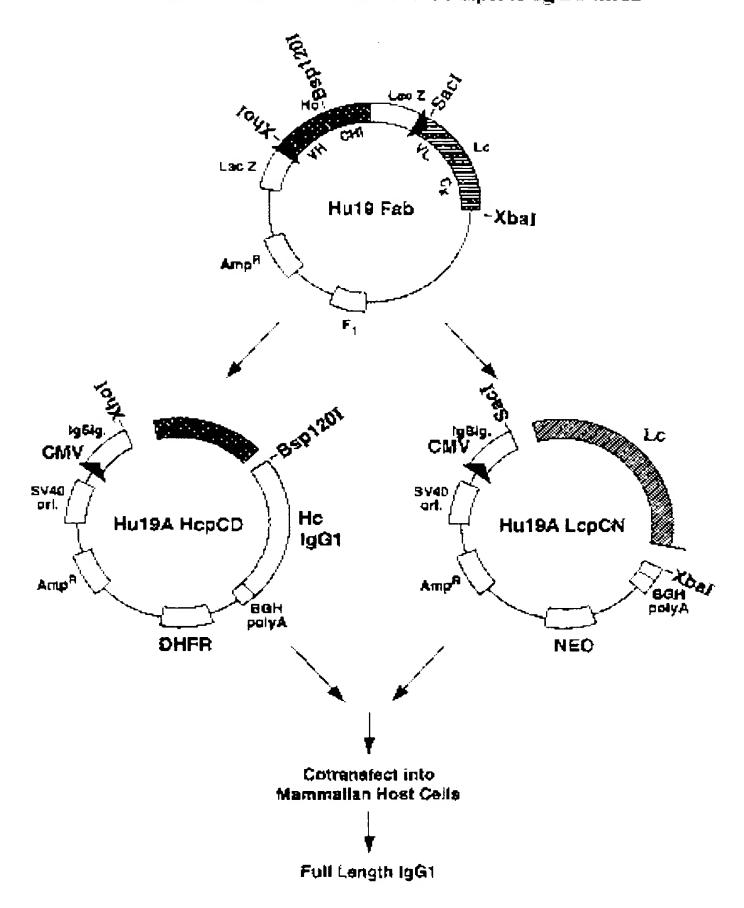
C (Continue	tion). DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	CROWE et al. Recombinant human respiratory syncytial virus (RSV) monoclonal antibody Fab is effective therapeutically when introduced directly into the lungs of RSV-infected mice. Proc. Natl. Acad. Sci. USA. February 1994. Vol 91. pages 1368-1390. See entire document.	1-11 and 14-16
A	WALSH et al. Protection from respiratory syncytial virus infection in cotton rats by passive transfer of monoclonal antibodies. Infection and Immunity. February 1984. Vol. 43. No. 2. pages 756-758. See entire document.	1-11 and 14-161-
A	WALSH et al. Analysis of the respiratory syncytial virus fusion protein using monoclonal and polyclonal antibodies. J. Gen. Virol. 1986. Vol 67. pages 505-513. See entire document.	1-11 and 14-16
A	BARBAS III et al. Human monoclonal Fab fragments derived from a combinatorial library bind to respiratory syncytial virus F glycoprotein and neutralize infectivity. Proc. Natl. Acad. Sci. USA. November 1992. Vol 89. 10164-10168. See entire document.	l-11 and 14-161- 11 a
A	SIBER et al. Comparison of antibody concentrations and protective activity of respiratory syncytial virus immune globulin and conventional immune globulin. Journal of Infectious Diseases. 1994. Vol 169. 1368-1373. See entire document.	1-11 and 14-16

Form PCT/ISA/210 (continuation of second sheet)(July 1992) *

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FIGURE 1

Conversion of Hu19 Fab to a Complete IgG1 mAb



2/31 Figure 2 Comparison of the Heavy Chain Amino Acid Sequences of various Hu19 mAbs

* * GL DoSB: EVOL VESGGGLYOPGGSLRISCANSGPTPS 30 IBA: MCWSCIILFLVATATOVHS......LE-R------T-L-19B: 19C: 1910: CDRI CORZ GL Dp5R SYEMNWVRQADGKGLEWVSYISSSGSTIYYADSVKGRFTISROWAXNSLY ខា IDA 19**B** TUC: (9D); ·-------Q-8------CDR3 GL: Dp58 LOMNSTRAEDTAVYYCAR 98 (SEQ ID NO: 4) 19A: ----T-----TAPIAPPYFDHWCQCTLVTVSS (SEQ ID NO: 5) tyb: (SED ID NO: 6) 19C: (SEQ 1D NO: 7) 1917: (SBQ ID NO: B)

3/31 Figure 3 Comparison of the Light Chain Amino Acid Sequences of various Hu19 mAbs

A. Leader and Variable

	CDR1
GL Dpk9:	DIQMTQSPSSLSASVCDRVTITC rasceis 30
·	MGWSCITLFLVATATEVES EL
•	MRVPAQLLGLLLWLRGARCDIQM
	⊂ยห2
GU Dpk9:	SYLWWYQQKPGKAPKLLIYAASSLQSGVPGRFGGGGGGTDFTLTISSLQP 80
19 A :	NELN E T T DTS
19 B ,C,D:	
	CDR3
GL Dpk9:	BDFATYYC * (SEQ ID NO: 9)
19A:	L-MQASINTPLFGGGTRIDMRR 105 [SEQ ID NO: 10)
19B,C.D:	
B. Con	stant Region (Cx)
	TVAAPSVFIFPPSDEQLKSGTASV/CLLNNFYPRBAKVQWKVDNALQSGN
19A.B:	
	SQESVTEQUSEDSTYSLESTLTLSKADYEKHKVYACEVTHQGLSSPVTRS
19 A, B:	············ <u>L</u>
Hu-K ₁ (9C ₁ D)	FNRCEC (SEQ ID NO: 12)
(9A,B	(SEQ ID NO: 13)

- 63003 10 01 630 111

WO 98/19704

4/31 <u>Figure 4</u>

Figure 4A-- DNA sequence of the plasmid Hull9AHcpcd

1	gacgtogoggecgetetaggeetecaaaaaageeteeteaetaettetgy	50
51	##tagntcagaggcgggcctcggcctataataaaaaaat	100
101	tagtcagccatycal.gggggggggaal.gggcggaactgggcggagttagg	150
151	ggcgggatgatgagcggqqcggqlactatgqttgctgactaattgag	200
201	alignatigelilitgeskanttnigentgetgggggggetggggggttteene	250
251	acctggttgctgactaattgagatgcatgctttgcatacttctgcctgc	300
301	ggggayddl.gggggadl.lla:macannohaapttgagagagattbogagaat	350
351	taatteeeggggategateegtegaegtaegaetagttattaatagtaat	400
401	caattacggggtcsttagttcstagconstatslggsgthongcgttaps	450
450,	taacttacggtaaatggcccgcctggctgaccgcccaacgacccccgccc	500
501	atligacylicae laaliyady halighlicone tagteacgocaategggactt	550
551	tocattqacgtcaatggqtgqactatttacggtaaactgcccacttggca	600
ธตา	gtacatcaagtgtateatatgeeaagtacgcccctattgacgtcaatga	650
653	nggtaaatggnnogontggnattatgcnoagtacatgaccttatgggact	700
90 3 .	thoptechtygnegtenetotangtattngboatogotattaonatggtg	750
751	al acceptible occasilace incestoggest anatamognit tracticaes	800

ខ្មា	gggatttecamgtstecamosocomttgamgtcamtgggmagtttgttttggc	850
851	accaaaatcaacgggacttbuddaaaklglughaacaachengeconattg	900
901	acgematggggggtaggeqtgtacggtggggggtctatataageagagd	950
	Eco Ri	
951	Egggtacgtgaacglougutogentggagacgccatcgaattotgagca	1000
1001	cacaggacotcacc <u>atq</u> ggatgqagetgtateateeUcUbcUbggUngon M G W S C I I L F L V A	1050
	heador start	
1051	acagetacaggtgtccactecgaggtccaactgctcgaggaglctggggg T A T C V H S <u>E V Q</u> L L B V - (SEQ ID Processed N-term,	1100 NO: 15)
כסגו	aggeotggteaggeotggegggtecctaagactetegtgtgtageagcetety	1150
1151	gaaccaccctcagtggctalaccalgoacbgggtccgccaggctccaggg	1200
1201	anggggetggagtgggteteateeattactggaggtagcaacttcataaa	1250
1251	ctackcagackcagkgaagggccgabtcaccabctccagagacaacgcca	1300
1301	agaactcactttatctgcaaatgaacagcdtgadagdddagggaggadaggd.	1350
1351	gtotattattgtgogacogococtatagcacogocotactttgaccactg	1400
1401	yggnnagggaacuntggtcaccgtotcctnagcctccaccaagggcccat	1450
1451	agg tetterceetggesechteeteessgagesectetgggggesesgeg	1500

1501	ត្តភពស្តេចផ្លូកសម្ភាពសម្ព័ធ្ធសម្តេចស្រុកសម្រេសប្រជុំក្នុងសមត្តធ្វើស្តេចផ្លូវប្រជុំប្រជុំ 	1550
1551	ptqqaactcaggcqccctgaccaqcggcqtgcacaccttcccggctgtcc	1600
160)	tacagtectoaggaptetactocctoagcagcgtggtgactgtgccctcc	1650
1651	agoagdiligggdacodagaccilacatdigdaangtgaatbaacaggcocag	1700
1701	caacaccaaqqtqqacaagaaaqttqaqccaaatcttgtqacaaaactc	1750
1751	acaeatgeceancgtgeceageacetgaaeteetggggggacegteagte	1800
1801	tteetetteeeeeaaaaceeaaggaeaceetealgalk:leeeggseek:	1850
1851	tgaggteaeatgegtggtggtgaegtgageeaegaagaeeetgaggtea	1900
1901	adttcaactggtacgtdgacggcgtggaggtgcataatgccaagacanag	1950
1.951	индрудунудыднарындардард караддуулардыдарды сосоо	2000
2001	catectaeaccaaactaactaactaaataacaaaaaaaaa	2050
2051	помеська обласний при	2100
2101	gqqcaqeeccgaqaaccacaggtytacaccetgcccccatcccgggalya	2150
23.51	gotgaconngaacoaggicagootgacotgootggicaaaggotictate	330 0
2201	ски додили (.cgc.rg), думу фруга дерска будового долгового с	2250
2251	tiansagannaegenteengtigaligyantinnganggehentifikothinoteta	2300
2301	cagcaageteacegtgyacaagaynaggtggnagnaggggaangbottet	2350

2351	catgeteegtgutgeatgaggetetgeaeaaceactacacgeagaagage	3400
2401		2450
	S P G K * end of beavy dusic (SEQ ID	NO: 16
2451	actgtgoottetagttgocagecatetgttgtttgoccotecocogtgoc	2500
2501	Linehbysecotyggaaggbyccacteccactytectttectaatacaatg	2550
2551	${\tt aggaaattgcatcgcattgtcllgaglsgglglmshhmhahlcllgagggg6}$	2600
260).	ក្នុក្សស្ត្រីក្នុងក្នុងក្នុងស្ត្រីស្ត្រីស្ត្រីស្ត្រីស្ត្រីស្ត្រីស្ត្រីស្ត្រីស្ត្រីស្ត្រីស្ត្រីស្ត្រីស្ត្រីស្ត្	2650
2651	tgetggggstgeggtgggcholatygaannagntggggntoganagogct	2700
2701	ggatetecegatececagetttgettetelatttettatttgeataatga	2750
2751	gaaaaaaaqqaaaattaatttLaacaccaalLdaglagUlgaUlgagdaa	2800
2801	algogitgoossaasygatgottiagagacagigtictoigcacagataa	2850
2851	ggacaaacattattcagagggagtacccagagctgagactcctaagccag	2900
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2951	gatteegtagagecaeacetligglasgggecseholigoliescaeaggels	3000
3001	gagagggcaggagccagagcatataaggtgaggtaggatcagttg	3050
3051	otootoocatttgottotgocatagttgtgttgggaagettggatagettg	3100
31.07	gadageteagggetgegatttegegedamaettgaleggemateetmgegt	3150
3150	gsaggeligytaggattlibatennegetgneateatggttegaenattgaa	3200

3201	сіясжісяісяся у праводня прав	3250
3251	tacnobygochengeteaggaaegagtteaagtaetteeaaagaatgaee	3300
3301	acaacctcttcagi.ggaaggbaaacagaat.ct.ggtgattat.gggtaggaa	3350
3351	авсотдяттегосаттестдадаадаатедасерияанудасыдын Uta	3400
34117	alabaghtotoagtagagaactoaaagaaccaccacgaggageteatttt	3450
3451	cttgccaaaagtttggaligaliguullaagacttattgaanaannggaatt	3500
3.501	ggcangtanagtngacatggtttggatagtcggaqgcagttctgtttam:	3550
3551	aggaagccatgaatcaaccaggmmacclhagaptctttgtgacaaggatc	3600
3603	#tgcaggantttgaaagtgacacgtttttcccagaaattgatttggggaa	3650
3651	atataaaetteteeeagaatseedaggugl.col.cl.nhgagghepaggagg	3700
3701	нымынардратоварьатавдітідаадтотасядаадаадаадастансад	3750
3751	gaagatgettteaagttetelgeleeceleeleaagelatgeafitteat	3 B Ò O
3 8 11 7	##9accatgggacttttgctggctttagatcagcctcgactqtgccttc%	3850
3851	agttgccagccatctgl.l.gtbbgcccobcccccgtgccttccttgaccct	3900
3901	ядаадардссастсссастутский иссинациана тунуунын tyggat	3950
3951	egcattqtetqagtagggtgteattetattetgggggggtggggt	4000
4 007	gacagcaagggggaggattgcgaagacaatagcaggcatgctgggatgc	405B

4051	ggtgggttttatggesonegelggggclogategagtgtatgnetgegge	41 00
4101	ogogateoogtegagagettggegtaateatggtealægalgfilkontgt	4150
4151	gligesalligilseloogobososathoosoossoosacatacqagccggaagca	4 200
4201	taaagtgtaaagcotgggylgcdlsalgsglgagcbaactcacattaatt	425 0
4251	gogttgogeteactgocogetttocagtegggsascolgtoglycosget	4300
4301	gnattaatgaatoggoodaogogoggggagagggggtttgggtattgggo	4350
4351	gotottoogotootogotootgaetogolycgunogotogoto	4400
4401	aggagegegetekaegahakabakaggaggaataataaggttataaaga	4450
4451	м towggggatnacgcaggaaagaacatgtgagcaasayyccaycaнаауд	4500
4501	ccaygasccytassasgyddydyllydlygdythtttocataggotocgd	4550
4551	crecotgacgagcateacaaaaaategacgeteaagteagaggtggegaaa	4600
4601	cccgacaggactataaagateccaggcglibbcccccttggaagctcccteg	4650
4550	tgagototootgttoogagootgoogottacaggataaatgtaagaallk	4700
4701	atmonthngggнөgoghggngotthotoaatgoteacgetgtaggtatet	4 750
4751	cagtteggtgtaggtegttegelinnmagningggntgtgtgtagaacece	4800
4801	obattcagecogacogotgogocttatocgghaschshoghottgagtoc	4850
4851	www.gaceaggacttategecaetggeageagecaechggtaaceag	4900

4901	gattageagagegaggtatgtagg <mark>eggtgetaeagagttett</mark> gaagtgg1.	4 950
4951	qqcctaactacygctacs:lsgaaggacaglabbbggbatctgcgctctg	5000
5001	страалссаяттассттедрааваадаяттуртаўстеттдатисдукын	5050
5051.	жинимпринопрофессов под примения при примения при примения применя при применя при применя при применя при при	5100
5101	Сусусауьвававаруя I.e.Leanysagatecht.tyatottt.totaogggg	5150
5151	tetgaegeteagtggaaegaaaaeteaegttaagggabbtbggteabgag	5200
5201	allalinaamagyatottoacotagatoottttaaattaaaaatgaagtt	5250
5251	ttaaaroaatotaaagtatatatgaqtaaacttggtetgaeagttaeeas	5300
5301	tgcttaatcagtgaggcsccLatcLosgogabotgtotatttogttonto	5350
5351	catagttgcctgactoccogtogtgtagataactacgatacqqqagqqct	5400
5401	taccatctggccccaqtqctqcaatqatacqqqqagagadccaacgclcaccq	5450
5451	устисяватын теадраа тааа сеадсеадсеада ададасада дедсад	55 00
5501	aagtggtcctgcaactttatecgeetecatecaglaskatbaakkgtbgpp	5550
5551	gggaagetagagtaagttegeeagttaatagtttgegeaaegttytt	5600
5601	yomahtgahamaggaatagtgatgtoxogotegtegtttggtatggette	5650
5651	atteageleegyllenemmappatomaggegagttmemtgateccommtgt	5700
5701	tgtgcaassaagogglilagoboobboggbootoogatogttgtcagaagt	5750

5751	aughtggengeagtgttateacteatggttatggengenetgeataatte	5800
รคถา	In: Nachglina kgrinabnegtaaga tgot tibbolig tgan bgg tgag tao t	5850
851	caaccaaqtcattdtqagaataqtgtatgcqgcqaccqaqttgctcttqd	5900
5901	coggegteantaegggataataeeggegeeseatageagnaetttaaasgt	5950
5951	gobnahoakhggaasanghtothoggggngaasantotnsaggshottao	60 00
5001	egetgttgagateeagttegatgtaaceeactegtgcacceaactgatet	6050
505)	teageatetttäettteaeeagegtttetgggtgageaaaacaggaag	6100
5101	gcaawatgcoqcaaaaagggaatawgggcgacacggaaatgttgaatac	6150
តាភា	teataetetteetthteaatattattgaageattateagggttattgt	8200
6201	etembqagoggataeatatttqaatqtatbbaqaaaataaacaaatagg	62 50
62 5 1	- postecomposesti.Cocecossasautumosest - 6284 - (SEO ID NO):14)

12/31

Figure 4B-- DNA sequence of the plasmid Hu19ALopon:

1	gacgtegegggegetetaggeetecaaaaaageeteeteaetaettetgg	50
54	aatagotouguggoogaggootoggoototgoataaataaaaaaat	200
£01	tagtcagecatgeatggggeggagtaggeggaactgggeggagtlagg	150
151	дредруютдерация правод праста при	200
201	atgnatgntttgnatanttntgnntgngggagnntggggantttnuan	250
251	acctgqttgctqactaattqagatqcatgctttqcatacttctgcctgct	300
301	ggggagaptggggachttepacacoptaactgacacacattecacagaat	350
351	taatteeeggggategategategaegaetagttattaatagtaat	400
401	daatilanggggtostitagi.tostagoodatalat.ggagt.toogogtilada	450
451	taacttaeggtaaatggeengeetggetgaeegeeeaaegaeeeegeee	500
501	attligacy licaa leafigeogtaligeticoostegtiesogocealiagggactil:	550
551	tecattgaeqteaatgggtggaetatttaeggtaaaetqeecaettqgea	600
ልዐገ	ghacatoaagtgtaboatatgccaagtacgoccootattgacgtcaatga	650
651	ogglæstidosgladasigsbookististigebookstatigsbookstatis	700
701	ttectaettggeagtaeatetaegtattagteategetattaeeatggtg	750
751	atgeggttttggcagtacatcaatgggegtggatageggttttgactcacg	800

801	gggattteeaagteteeaeeeeattgaegteaatgggaagtttgttt	850
a51	Accessaticsacgggscull.commans.lgl.rgl.aacaanteoggcoonattg	900
901	acgcatatgggcggtaggcgtgtacggtggaggtctatataagcagagc	95 0
	Eco Ri	
951	Lgggtengtgescogtcagatogcotggagacgccatoganttornagoa	1000
1001	cacaggacetesec <u>atg</u> ggatggagetgtalestecheklekligglisges M G W S C I I L F L V A	1.050
	Leader start	
1051	acagetacaggtgtecaclongageleaconnaglelonalmolenel.glc T A T G V H S <u>E L T</u> O S P - (SEQ ID Processed N-term.	าากอ No: 18)
1101	tgcatotqtagqaqacagagtcaccatcactbyccgygcaaclcagag1g	1150
1.151	Ulaghaactthhhaaattggtateagcagaagecaggggaagccoctaeg	1200
1201	ntestgatetatgatgeatesasttegsaasytggggtsesstsasggtt	1250
1251	ину tygcagtygutetgggatggattteagteteaceateageagtetge	1300
1301	agectgaagatettgeaatgtattaclgtdaagcgaglatdaalacdddg	1350
1351	etttteяподда ддяшес адааtagatatga gacqaac tgtg qc tgcacc	1400
1401	Shetgtetteatettecegecatetgatgageagttgaaatetggaactg	1450
1451	nd Lakyttighg tigdetigaataactiota toocagagaggocaaagta	1500
1501	dagliggaaggliggataaogdootdoaatogggtanotoooagganagtist	1550

1551	сасаўаўсаўўасаўсваўўайаўсяйсінинуссфолуполусостіда	1600
ነሉዌነ	ngotgagoaaagoagactaogagaaacacaaagtotacycotycyнну)	1650
1651	addeslinaggynnigsgottgenogtnacamagagottemacamagagaga LPVTKSFNRGE	1700
1701	Xba I gtgttngtgagntgatectetaggteatetaegtatgateageellegae: C * end of light chain (SEQ ID NO: 19)	3750
1751	tgtgeettetagttgeeageeatetgütgb1.lgnaaptacoacgtgeett	1800
3.803	enthgameetggaaggtgeeacteccaetgtcctttcctaataaaatgag	1850
1851	qaaattgcatcgcattgtctqagtaggtgtcal:kattctggggggtgg	1900
1901	gy hygygangganngnangggggggggattggggaagacaatagcaqqcatg	1950
1951	ctgggggatgcggtgtatatggaaccagctggggologeoagotogag	2000
2001	diagontiligolitudosalibliokkahhbyrataatgagaaaaaaaggaaaa	2050
2051	ttaattttaacaccaattcaqtagttqattqaycaaalgogbbgcommaam	2100
21.01	aggatgotthagaganaghgttototgonoagat aaggacaaaca ttatt	2150
2151	cagaggyagtacccayagolgagack.cotaagoongtgagtggcacagca	2200
2201	ttotagggagaaatatgottqtoatoaoogaayoobgattoogtagagoo	2250
2251	acacettootaagggecaatetoeteadacaggalжумумумуучулуур	2300

2 D W L	entigggerverdettal statistalistet entiger verde en en en en en en	
8. 51	tholyacstaghlighghluggagonliggalogstocscostggttgasca	2400
2401	agatggattgeacgcaggttetecggecgettgggtggagaggetatteg	2 4 50
2451	getatgketgggeackaengaensteggetgetetgktgeegeegbytte	2500
2501	oggotgleagegeaggggggangglundddddglasagaeegaeetgte	2550
2551	oggtgeeetgaatgaaetgeaggaegaggeagegeggetatogtggetgg	2600
2603	imangaoggynglitnobhgognagotighgofinganghtigli@a@bgaagng	2650
2651	ggaagggaetggetgetattggggegaagtgeegggggaggateteetgte	2700
2701	allo losco bligotionligoogagaaay kalioostica liggotyatgoastyo	2750
2751	ggeggetgeataeget tgateeggetaeetgeeeattegaeeecaageg	2800
28 01	aaacstogcatogagogagoacgtactoggatggaagooggtotogtoga	3850
2851	teaggatgatetggaegaagageateagggggetegegageegaacteg	2900
2901	logocaggoloaaggogdatgdddgaogaoggogaggatotogtogtgaoo	2050
2951	catqqcgatqcctqcttqccqaatatcatggtggaalatqgccqcttttc	3000
3001	tggattcatognotgtggocggotgggtgtggoggaoogotahouggaoa	3050
3051	tagogttggntadongtgatattgdtgaagagntbggdggaa1ggaal	3100

2101	Butter de tres en mande en en en mande en en mande en mande de men	3130
3151	egeettetategeettettgaegagttetLulgugogggaptotggggtt	3200
3201	eganatgacegaecaagegaegeceaaectgecateaegagatttegatil.	3250
3251	ncaecgingdef.Lelalgasaggthgggetteggsategtttteegggae	3300
3301	geeggetggatgateeteeagegeggggabebesshigeslygsagthettege	3350
3351	concoopactigtthattgcagottataatggttacaaataaagcaata	3400
3401	gcatcecasattcacasataangcattththhhppotgcattctagttgt	3450
3451.	ggtttgtocaanotontonatgtatettateatgtetggategeggeege	3500
3501	gatecegtegagagettggegtaalealiggloahagntgtttcctgtgtg	0550
3551	ини t.tgttatoogotoooootatooooooooooooooooooo	3600
3601	agtqtaaaqeetgqqqtqeetaatgaqtqagdbaachdaddhiaddqqq	3650
3651	htgagatagatgacagattoaggagagagatgtagtgcagatgca	3700
3701	ttaatgaateggeeaacgegeggggagagggunlikgngtattgggeget	3750
3751	cttoopottootogotoaetgactogotgagetaggtaggtaggtaggg	3800
3800	cyagoggtatnagotoaxaggoggtaataeggttatecacayaate	3850
3851	адудуы),напусадувандансабуфдадсаааддосадсаааддоса	3900
3903	ossancolasssaoscomo Llochquest bitteestagseteeseee	3950

3951	cotgacqaqcatcacaaaaatcqacqctcaaqtcaqaqqlqусqыныско	4000
4001	ganaggantataaagataobaggogtttoboootggaagetoootegtge	1050
4051	geteterligbbodgaudeligeognillacografiaontghoogeofitiete	41,00
4101	cottegggaagestggegettteteaatgeteaegetgtaggtateteag	4150
4151	Linggtgtaggtngttegeteeaagetgggetgtgtgeacgaacceeccg	4200
4201	tteageeegaeegetyegodluuluugglusotaloghothgagboeaee	4250
4251	coggitangacangacttatogodactggdagdagdactgdtaadaggat	4300
4301	tagcagagcgaggtatglaggogglydlanagaghtdttgaagtggt	4350
4351	nhaectacggotacactagaaggacagtatttggtatetgegototgetg	4400
9401	aagccagttaccttcggaaaaagagttggtagnlctlgalccggcaaaca	4450
ú ú51	saucanngchggtagngghggttttttttttttgtttgcangcagcagettnege	4500
4501	gengaaaaaaggateteaagaayateetttgatektiitelangggytel	4 550
4551	gangotoagtggaeogaaanotonogttangggatttttggtontgagatt	4600
1601	atcsaaaaggetetteacnisgainellillsaattasaastgeegtetta	4650
4651	antcaatctaaagtatatatgagtaaacttggCctgacagUtaccaatgc	4700
4701	htmatemytgmggcaectatetemgegatetytetmttegttemteemt	4750
4751	agttgootgactccccgtcgtqtagataactacgatacggqagggcttac	48 00

i Ali i	racringabrocadrabandosarias cadadadada accade cadadada c	4820
1851	ccagatttatcagcaataaaccagccagccygaayggccgaycycayaay	4900
190)	tygtectgeaactttateegeetecatecagtetattaattgttgeeggg	4950
1951	aayotayaylaayl.liigoosghtaahagtitgoogoaacghtylitgoo	5000
5001	attgetacanneategtggtgteacgetegtegtttnggtatggetteatt	5050
5051	cagobooggttoccaacgatonaggogagttacatgatccccontgttgt	5100
5101	gcaaaaaagcggttagctddttdgglddloogaldgllgloagaaglaag	5150
5151	libygangaagtgtbataantaattatggcagcactgcataattatat	5200
5201	tactgtcatgccatccqtaagatgcttttctgtgaclggl.gagtactcaa	5250
5251.	cusaginalindgayaatagtgtatgqggqgacogagttgctcttgcqcq	5300
5301	gcgtriatacgggataataccgcgccacatagcagaactttaaaaqtgct	5350
5351	calicalitggaaaangliliniitnggggngaaaactotcaangatottacngo	5400
5401	tgttgaqatccaqttcgatgtaacccactcgtgcacccaactgatcttca	5450
5451	gratettttaettteaecagegtttetgggtgageaaaaacaggaaggea	5500
5501	авитуссодиванна дудин тинуддодира од да а тутту се	5550
5551	tactntbcctttttcaatattaktgaagnalillalmagggllaltigtelm	5600
5601	atgagcggatacatatttgaatytallilagaaanalanacanalagyggf.	5650
5 65 1	teegegeacattteeeegaaaagtgeeaeet 5681 (SBD ID NO: 17	'1

Figure 4C Heavy chain coding sequence in the plasmid Ha19BHeped

	Eco Hi	
	<u>ក្នុងនៅដែលពួច</u> ដុំងុំជុំជុំ	1000
1001		3.05.0
1001	Brastttaggetgagetgggtttteetegtggetetttaagaggtgt M E F G L S W V F L V A L L R G V	1050
	Leader start	
1051	odagtgtenggtgengetggtggagtetgggggaggecliggtmaggectg	1100
	Q = Q = Q = Q = Q = Q = Q = Q = Q = Q =	
	Processed N-term	
1301	gogggtosstaagaststogtgtgsayssludggaansassotsagtggs	1150
1151	tatadosi.gusdi.gggtooggoosggotocangga aan gg y dtgyagtgyg).	1200
3.2 01	otoatocattaotggaggtageaacttealaaachantcageotoegtga	1250
1251	agggoogabboaccalchumagagacaangecaagaactcactttatolg	1300
1301		1350
12111	oakatgaacagootgacagoogacgaacacggctglobabbatbgtgcgac	1330
1351	egadaalalagaadogadotaotttagadoaotgggggeeagggsaeaddigg	1400
3403	tencentetecteagectecaccaagggedesLeggtetteccectggca	1450
1451	dontontonaagagoanototggggggaaaagaggaaatgggutgootggt	1500
15 01	caaggan lankkunnogaaqonggtgaqogtgtogtggaactdaggdgdoo	0550
A F # 4		7545
1551	tgandaguggdgtgdawedebboonggdbgtdotadagtddtdagganbo	1600

1601	tactecoteageagegtggtgaccqtgccctccagcagcttgqgcaccca	1650
1651	gaddiacaidigdaadgigaaldanmagdhogmagnaanananaaggiggada) 70 0
1701	nganagttgagecenaatettgtgacaaaactencacatgeceneegtge	1750
1751	ccageachtgaacteotggggggggeegtcagtcttcctcttcccccaaa	1800
1801	acceaaggacacceteatgateLoneygsonechyaggtopnatgrybgg	1850
1851	tggtggaegtgagecocgaagaccctgaqqtcaagttcaactgqtacgtg	1900
1901	gacgqcqtygaggtgcataatgccaagacaaagccgcgggaggaggta	1950
195)	naanagozogtacogggtggtcagcgtcctcaccgtcctgcaccaggact	2000
2001	ggctgaatggcaaggagtacaagl:graaggtotoooanaanaagcootoona	2050
2051	данасовта далавасовтатос алад солладделдесс еда даасс	2100
2101	acaggtgtacaccctqccccatcccgggatgagdlgaccaagaadcagg	2350
2151	tougestgasstgastgassaggettstatessagsgasategesqtg	2200
2201	gagtgggagagcaatgggcagdoggagannaadlacaagagpgcgnnhpc	2250
2251	ngigoiggactoogacggctccttcttcctctacagcaagctcaccgtgg	2300
2301.	ncangagnaggaggaacgtottotoatgctcogtgatgcat	2350
2351	gaggelmlycacaaccaelacaggggggggggggggggggggggggggg	2400

2001 tasatyahagataho - (SPQ TD NO:20)

K * end of heavy chain

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Figure 4D Light chain coding sequence in the plasmid Hu19BLopen

	Eco RI	
	goattocatgga	1000
ו ממר	eatgagggtecegeteagetectagggetectgetgetetggeteegag M R V P A Q L L G L L L 7. W J R	1050
1051	Loader start gtgccagatgtgacatacagatgaccccagtcbccatcccccctgtctgca	1100
• • • • • • • • • • • • • • • • • • • •	G A R C <u>D I Q</u> M T - (SPQ ID No: 23) Processed N-term	2100
1101	tetglaggaganagaghnacnatoznttgneggggeaacteagagtgtlag	1.1 50
11.51	tamotttttammttggtatcagcagamgccmggggmmgcccctdcc	1200
1201	tgatatal.galgdal.ccarbbogdaaagtggggtdodatdaaggttoagt	1250
1251	ygnagtggatotgggatggatttcagtctcaccatcagdaybolgnagon	1300
1301	tgaagatettgessiglailantghossgogagtatoaataooogettt	1350
1351	Loggoggzagggzocagzatagatatgagacyaactiglygolgceccetot	1400
1301	gtatbenlebbnoogonahetgatgageagttgaaatatggaaatgeata	1450
1451	tgttgtgtgcctgctgaataachtobatcocagagaggccanagtacagt	1500
1501	gganggtggataacgccctccaatcgggtaadkommaggagagtgtcaca	155 0
1551.	ундоходдарадоходдарадсарствеадсетсяўснусную у принцикацию	1600
1601	фицомалисадария под прадеждения по при	1650

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1651 atcagggcctgagcttgcccgbcanesagagcttcaacaggggagagtgt 1700

L P V T K S F N R G F C (SEQ NO: 24)

Xba I

1701 <u>tag</u>tgagstgatcc<u>tmlaga</u> (SEQ ID NO: 22)

end of light chain

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Figure 4B. Heavy chain coding sequence in the plasmed Ho19CHepod

	Eco RI	
	gaattoggtacc	1000
1001	atggagtttggggtgagctgggttttcctcgtggdtdttttaagaggtgt	1050
	MEFGLSWVFLVALLRGV	
1051		1100
1001	Q C Q V (0), V - (SEQ ID NO: 21)	1100
	Processed N-term	
ומור	gogggtesstæagastetegtgtgeægestetggeaddeookkaagliggd	1.15a
1151	tataccatycactyggluccyncwygotocagggaaggggatggagtgggt	1200
1201	eteatecaltschugegytageaantteataaactacgcagactcagtya	1250
	S N F I N Y <u>A</u> (SEQ ID NO	: 26]
1251	agggcogattoaccatetecagagacaacgccaagsacheachhahchy	1300
1301	casatysacayoolyscsgcngsggscscggctgtctattattgtgcgac	1350
1351	ogodootatagoaoogodotactttgaccactggggcdagggaaacccl.gg	1400
1401	Lastinghoundhou g collectedangggeeealeggtelleceeelygea	1450
1451	eachast as a sayreachtaí promover son sugarachtaí tagairt sa tagair	15 0 0
1421	ecctectecaagageacdkallyggygusasguggucotuggutusotugt	1500
1,501	chaggactacttecccqaaccggtyaccgtyloglggmaclosgggggcc	1550
1551	bgaccageggegtgeaeacetteacggetgteathcaglcolubggbolc	1600
1.601	tantoontoognagegtggtgaeegtgeenteeageagettggggeaceau	3.650

1651	унговаратовортураторого адрагае савуулуунга	1700
1701	agaaagttgageedsaal.dl.lglyscaaaataacacatgeecaccgtge	1750
1751	coageacotgaactcetggggggaccgteagitebileclebbrecopposas	1800
1801	aciidaagyanachotoatgatotoooggacoootgaqgteacatgegtgy	1850
1851	tqqtqqacqtqaqccacgamgmuuul.gmggtnamqttomactgqtacqtg	1900
1901	васвводтпваддтроатаатдестарастаадестреуурадуад:ндра).950
1951	Caacagcacgtaccgggl.ggt.cagpgtoctcaccgtcctgcaccaggact	2000
2001	gyotgaatggcaaggagtacaagtgcaaggtctccaacaaaggcccLucca	2050
2051	gcccccategagaaaacccuulululuuaaagggggggggggggggggg	2100
2101	#cayytytanacontgonoonatecogggatgagetgaecaagaaccayy	2150
2151	teagectgacctgcctggtcassggctlchsluncagogacatngcogtg	2200
2203.	уму крудадароми крудсадсоддараасаастасаадассасусство	2250
2251	cqtqctqqactccqecqycluml:lmthccbntanagnaagctcaecqtqq	2300
2301	acaagagcaggtggcagcaggggaacgtcttdtdalydhecghgytgcat	2350
2351	gaggototgonomacomotacacacacagaagagactatacatgtataayyy	2400

2401 Lametgatagatato - (SEQ ID NO: 25)

K * pod of beavy chain

Figure 4F Heavy chain coding sequence in the plasmid Hu19DHeped

	Eco Ri	
	<u>gaelloggtao</u> c	1000
רחמו	Atggagtttggggtgagetgggtttteetegtggetetttaagaggtgt M E F G L S W V F L V A L L R G V	1050
1051	ccagtgtcaggtgcagctggtggagtctgggggaggcnhgghcaggcotg Q C <u>D V O</u> L V - (SEQ ID MO: 21) Processed N-term	1100
1101	yngyghennhaagantotegtgtgcagoototggaaecacecteagtggc	1150
1151	tataccatgcactgggtccqccaqgctccayggamygygklygmg%gggt	1200
1201	ctcatccattactygagytagdaanlinalagaatactpagantcagtga	1250 O: 28)
1251	нуудаадаttaaccatataagagacaacgocaagaactcactttatctg	1300
1301	caaatqaacagcctgacagccgaggacacggctgtclal.lat.l.gl.gcgac	1350
1351	ngenactatageacogecotantttgaecantggaggecagggaaccctgg	1400
1401	tcaccgtctcctcagectccaccaagggcccaloggtcttccccctggca	1450
1451	ocotootocaagagcaeptotgggggaacagcggccctyggctgcctgg1.	1500
1.501	naaggantacttoooogaacoggtgaoogtgtoqtgqaactcagqqcgccc	1550
1551	Lyandagoggogtgoedandttoooggotgtodtacagto ct caggadtd	1600
1601	Lantocotoagoagogtggtgacogtgccctocagcagcttggggcaccca	1650

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1651	дасстасатстдеаасдідавіснюяндородового обрадіддаса	7,000
1701	Agasayllgaynocaeatottgtgacaaaactcacadalyoogagogtgo	1750
1751	colgeacetgaaclee.ggggggacogteagtetteetetteecceeeaaa	1.800
1801	annnaaggacacceteatgatetennyyannnetgaggtezeatgegtgg	1850
1851	tggtggangligagcoscgssgscoctgaggtcaagtLosanligghacgtg	1900
1,901	naciticatédagétács/ws/idicadacadacaditécitédédagédagédagite	1950
1951	C84C49040gtanogggtggtgatcatcatcatcattatatacatgact	2000
2001	ggotgaatggcaaggagl.неннуtgcaaggtotocaacaaagccctcccн	2050
2051	gcccccalngegaaaaccatctccaaaqccaaaggynegncongegaacc	2100
21.01	anaggtgtacaccctgcccccalumogggatgagctgaccaagaaccagg	2150
2151	tragrolgadolgnotggtopooggettetateecagegaeslugeegtg	2200
2 2 01	аяврайцай в в в в в в в в в в в в в в в в в в в	2250
2251	CGTGG Lygantoggacggctccttcttcctctacaggaagnlcannghgg	2300
2301	acaaqagcaggtggcag::>ggggaacgtetteteatqeteegtqatgea1.	2350
2351	умядототдевсвассастаннеу нужнумядостогостятстесядду	2400

2401 Leasigntagatate (SEO ID NO: 27)

K * end of heavy chain

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Figure 4G Light chain coding sequence in the plasmid H19CLopen

	Siction PiT	
	gaattocatgga	1000
1001	a <u>etg</u> egggtaaaagataegataategggataatgatgatatggataageg	1050
	MRVPAQIOGOLULWLK	
	Leader start	
1051	gtgddagatgtgadatedagatgmoungtotoodtoodtgtotgda	1100
	G A B C \underline{D} I \underline{Q} M T - (SEQ ID NO: 23)	
	Processed M-term	
1101	totgtaggagacagagtoscostomettgccgggcsactcagagtgttag	1150
1151	taactttttaaattggtatcagcagaagccaggggaagcccchacgctcc	3200
1201	Laukakatan kanatan merekan menekan gatan pertengan teratuan di	1250
12(/1	Lgutchatgehgestersettegessegtggggtereateaangtteant	1230
1351	ggcagtggatetgggatggattteagteteaecateageagtetgeagee	1300
1301	Lgaagatottgcaakglabtachgtmaagogagtatoaatacoocgottt	1350
1351	teggeqgagggaeeaqaatagatatgaqaegaaetgtgggdtgeaeealct	1400
1401.	gtottentetteengenatotgatgageagttgaaatetggaaetgeete	1450
1451	lig lingligtgooligeligesheedt totahoodagagaggooddagtadagt	1500
1501	ggaagytggataacgccctccsatmgggleachcccaggagagtgbcaca	1550
		• •
1551	gagdaggadagdaaggadagdaddtadagdddl.tagnagdaccoblgangdt	0.600

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 $1601-{\tt gagoalageagactacqagaaacacaaaqtctacgcctgcgaaqtcaccc} = 1.650$

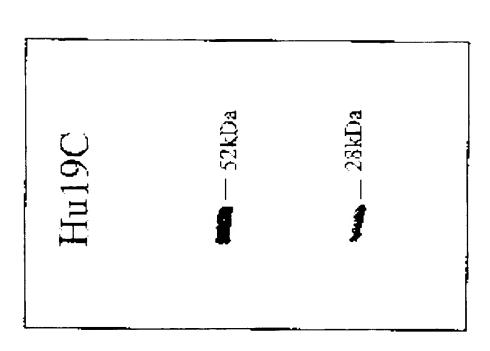
1651 atcagggcatgagatggcacgloscommagagalttrannegggggagagtgt 1700

 $\underline{\mathbf{S}}$ PVTKSPTRGQC(SEQ NO: 30)

Xba I

170). tagtgagatgatoc<u>totaga</u>tetaegtatgateagcetegaetgtgcett - (SEQ NO: 29)
* und of light chain

ESPECTIVELY UNDER REDUCING CONDITIONS Fig. 5. COOMASSIE STAINED SDS-PAGE GEI ANALYSIS OF 10UG HU19B AND HU19C



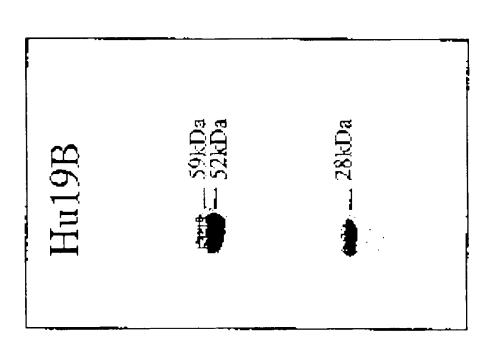


Fig. 6. SEPARATION OF HU19B GLYCOVARIANTS BY ANION EXCHANGE CHROMATOGRAPHY

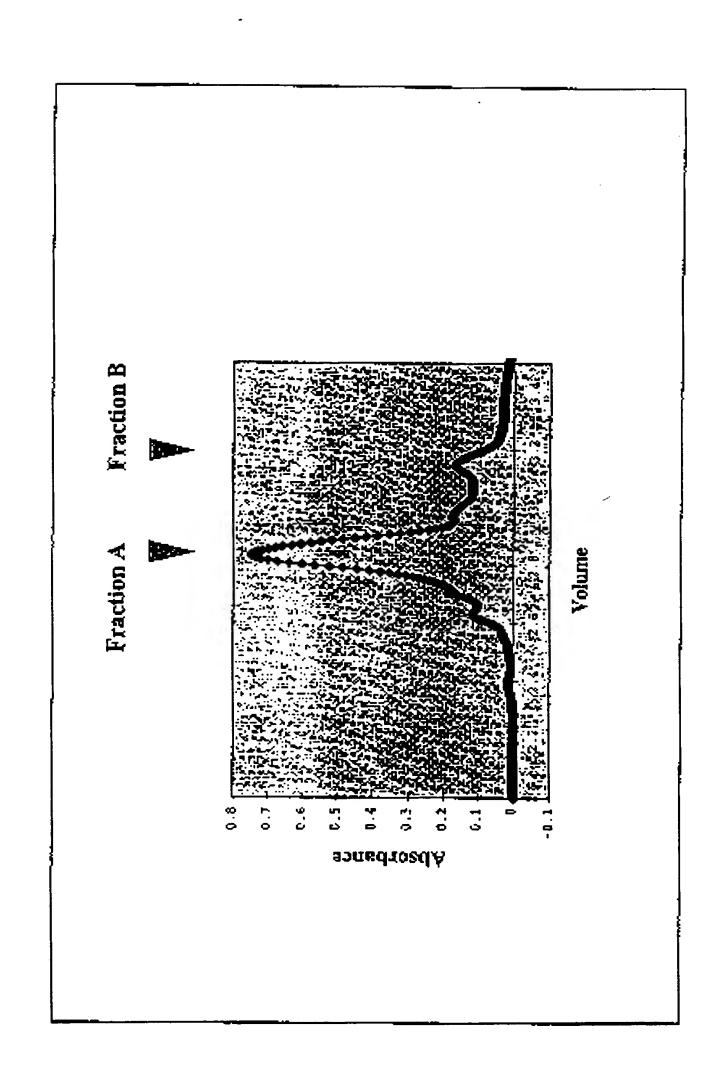


Fig. 7. SDS-PAGE of 3lycosylation Variant of Hu19B

Glycovariant

Mormal FAb

		•
		g r i